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 * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *
=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull
=> e sadziene ariadna/au
E1        87   SADZIENE A/AU
E2         3   SADZIENE ADRIADNA/AU
E3        35 --> SADZIENE ARIADNA/AU
E4         1   SADZIK E/AU
E5         1   SADZIK E S/AU
E6         1   SADZIK KAZIMIERZ/AU
E7         1   SADZIK PAWEL/AU
E8         4   SADZIK ZDZISLAW/AU
E9         5   SADZIKAVA N/AU
E10        6   SADZIKAVA NADYA/AU
E11       44   SADZIKOWSKI A/AU
E12       27   SADZIKOWSKI A B/AU

=> s e1-e3 and borrel?
L1       124 ("SADZIENE A"/AU OR "SADZIENE ADRIADNA"/AU OR "SADZIENE ARIADNA"
          /AU) AND BORREL?

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2        34 DUP REM L1 (90 DUPLICATES REMOVED)

=> s l2 and ((13 kilodalton?)or(13 kda)or(13,000 dalton?))
L3         5 L2 AND ((13 KILODALTON?) OR(13 KDA) OR(13,000 DALTON?))

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 5 ANSWERS - CONTINUE? Y/(N):y

L3  ANSWER 1 OF 5  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN  2001:570667  BIOSIS
DN  PREV200100570667
TI  Methods and compositions including a 13kD B. burgdorferi protein.
AU    ***Sadziene, Ariadna***  ; Barbour, Alan G.
    ASSIGNEE: Board of Regents, The University of Texas System
PI  US 6300101 October 09, 2001
SO  Official Gazette of the United States Patent and Trademark Office Patents,
    (Oct. 9, 2001) Vol. 1251, No. 2, pp. No Pagination. e-file.
    ISSN: 0098-1133.
DT  Patent
LA  English
AB  All  ***Borrelia***  burgdorferi sensu lato isolates characterized to
    date have one or a combination of several major outer surface proteins
    (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with
    polyclonal or monoclonal antibodies at a frequency of 10-6 to 10-5. One
    mutant that lacked OspA, B, C and D was further characterized in the
    present study. It was distinguished from the OspA+ B+ cells by its (i)
    auto-aggregation and slower growth rate, (ii) decreased plating efficiency
    on solid medium, (iii) serum- and complement-sensitivity, and (iv)
    diminished capacity to adhere to human umbilical vein endothelial cells.
    The Osp-less mutant was unable to evoke a detectable immune response after
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    the same duration as wild-type cells. Polyclonal mouse serum raised
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AU ***Sadziene, Ariadna*** ; Barbour, Alan G.

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IT Major Concepts
Human Medicine (Medical Sciences); Methods and Techniques

IT Chemicals & Biochemicals
13 ***kiloDalton*** ***Borrelia*** burgdorferi sensu lato protein

IT Methods & Equipment
13 ***kiloDalton*** ***Borrelia*** burgdorferi sensu lato protein production method: production method

L3 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 2001:538523 BIOSIS

DN PREV200100538523

TI Methods and compositions including a 13kDa B. burgdorferi protein.

AU ***Sadziene, Ariadna (1)*** ; Barbour, Alan G.

CS (1) San Antonio, TX USA
ASSIGNEE: Board of Regents, The University of Texas System

PI US 6296849 October 02, 2001

SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 2, 2001) Vol. 1251, No. 1, pp. No Pagination. e-file.
ISSN: 0098-1133.

DT Patent

LA English

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IT Major Concepts
Biochemistry and Molecular Biophysics; Infection

IT Chemicals & Biochemicals
13kDa ***Borrelia*** burgdorferi protein; monoclonal antibodies; outer surface proteins: functional aspects

ORGN Super Taxa
Spirochaetaceae: Spirochaetales, Spirochetes, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
Borrelia burgdorferi (Spirochaetaceae): pathogen

ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

L3 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 1995:220154 BIOSIS

DN PREV199598234454

TI ***Borrelia*** burgdorferi mutant lacking Osp: Biological and immunological characterization.

AU ***Sadziene, Ariadna*** ; Thomas, D. Denee; Barbour, Alan G. (1)

CS (1) Dep. Med., Univ. Texas Health Sci. Cent. at San Antonio, San Antonio, TX 78284 USA

SO Infection and Immunity, (1995) Vol. 63, No. 4, pp. 1573-1580. ISSN: 0019-9567.

DT Article

LA English

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osps). Mutants of B. burgdorferi lacking Osps were selected with polyclonal or monoclonal antibodies at a frequency of 10-6 to 10-5. One mutant that lacked OspA, -B, -C, and -D was further characterized. It was distinguished from the OspA+B+ cells by its (i) autoaggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum and complement sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell

immunization even though mutant survived in mouse skin for the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two types of monoclonal antibodies (MABs) with growth-inhibiting properties for mutant cells were identified. The first type bound to a ***13*** - ***kDa*** surface protein of B. burgdorferi sensu stricto and of B. afzelii. The MIC of the Fab fragment of one MAb of this type was 0.2 mu-g/ml. The second type of MAb to the Osp-less mutant did not bind to B. burgdorferi components by Western blotting (immunoblotting) but did not bind to unfixed, viable cells in immunofluorescence and growth inhibition assays. These studies revealed possible functions Osp proteins in ***borrelias*** , specifically serum resistance, and indicated that in the absence of Osp proteins, other antigens are expressed or become accessible at the cell surface.

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ORGN . . .
Mammalia, Vertebrata, Chordata, Animalia; Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Spirochaetaceae: Eubacteria, Bacteria

ORGN Organism Name
human (Hominidae); mouse (Muridae); ***Borrelia*** burgdorferi (Spirochaetaceae)

ORGN Organism Superterms
animals; bacteria; chordates; eubacteria; humans; mammals; microorganisms; nonhuman mammals; nonhuman vertebrates; primates; rodents; vertebrates

L3 ANSWER 4 OF 5 LIFESCI COPYRIGHT 2003 CSA

AN 2002:49123 LIFESCI

TI Methods and compositions including a 13kDa B. burgdorferi protein

AU ***Sadziene, A.*** ; Barbour, A.G.

CS Board of Regents, The University of Texas System

SO (20011002) . US Patent: 6296849; US CLASS: 424/141.1; 424/1.49; 424/150.1; 424/164.1; 424/184.1; 424/234.1; 435/7.32; 435/69.3; 530/825.

DT Patent

FS W2

LA English

SL English

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10 super(-6) to 10

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AU ***Sadziene, A.*** ; Barbour, A.G.

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UT Antibodies; Patents; OspA protein; OspB protein; OspC protein; OspD protein; ***Borrelia*** burgdorferi

L3 ANSWER 5 OF 5 USPATFULL

AN 2002:140866 USPATFULL

TI Methods and compositions including a 13 kD B. burgdorferi protein

IN ***Sadziene, Ariadna*** , Nutley, NJ, UNITED STATES
Barbour, Alan G., Irvine, CA, UNITED STATES

PI US 2002071847 A1 20020613

AI US 2001-973406 A1 20011009 (9)

RLI Division of Ser. No. US 1994-264036, filed on 22 Jun 1994, PATENTED
Continuation-in-part of Ser. No. US 1993-79601, filed on 22 Jun 1993, PATENTED Continuation of Ser. No. US 1992-924798, filed on 6 Aug 1992, ABANDONED Continuation of Ser. No. US 1989-422881, filed on 18 Oct 1989, ABANDONED

PRAI DK 1988-5902 19881024

DT Utility

FS APPLICATION

LREP FROMMER LAWRENCE & HAUG, 745 FIFTH AVENUE- 10TH FL., NEW YORK, NY, 10151

CLMN Number of Claims: 30

ECL Exemplary Claim: 1

DRWN 5 Drawing Page(s)

LN.CNT 1349

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins

(Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10.sup.-6 to 10.sup.-5. One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA.sup.+B.sup.+ cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 .mu.g/ml. Class B mAbs did not bind by Western Blot to B. burgdorferi cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistance, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.

IN ***Sadziene, Ariadna*** , Nutley, NJ, UNITED STATES

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SUMM . . . of basic information about all spirochetes. The spirochete cell is unique in several aspects (33). One of the features of ***borrelia*** is the abundance of one or several lipoproteins in the outer cell membrane (16, 19, 20, 34, 43). Much has. . . been learned about immunogenicity, as well as biochemical and genetic aspects, of these lipoproteins in Lyme disease and relapsing fever ***borrelias*** (4, 16, 19, 35, 37, 42, 64).

SUMM [0004] The lipoproteins OspA and OspB are major contributors to antigenic distinctness of Lyme disease ***borrelias*** (6). Both OspA and OspB are co-transcribed from a single operon located on linear plasmid of 49 kb in B.. . .

SUMM . . . The findings of Cadavid et al indicated that differences in invasive properties and tissues tropism between serotypes of related spirochete ***Borrelia*** turicatae, a relapsing fever agent, may be determined by the expression of a single surface protein that is analogous to. . .

SUMM . . . Osp proteins (51, 54). Our first intent was to characterize morphology and function of the Osp-less mutant. We asked whether ***borrelias*** lacking OspA, B, C, and D would be altered in such functional properties, as (i) generation time, (ii) ability to. . . potential to evoke immune response after intradermal live cell inoculation, and (vi) ability to survive in the skin. Among pathogenic ***borrelias*** the role of surface lipoproteins in these respects

have not yet been reported.

SUMM . . . showed the presence of a major low-molecular-weight lipoprotein specific for B. burgdorferi and raised the possibility that it was a ***borrelial*** equivalent of Braun's lipoprotein (36). Another study reported an immunogenic 14 kDa surface protein of B. burgdorferi recognized by sera. . .

SUMM . . . and B. garinii strain Ip90 (1, 17). B. hermsii HS1 serotype 33 (ATCC 35209; ref. 11) was abbreviated to Bh33. ***Borrelias*** were grown in BSK II medium and harvested by methods described previously (3, 5). When culturing tissues from animals, rifampicin. . . (25 .mu.g/ml) were added to the medium. Cells were counted in a Petroff-Hauser chamber by phase-contrast microscopy. In some experiments ***borrelias*** were also grown on solid BSK II medium as described (32, 51). To estimate growth rate, ***borrelias*** at an initial concentration of 2.times.10.sup.6 cells/ml, were grown in tightly capped, 13.times.100-mm polystyrene culture tubes (Falcon Labware, Lincoln Park,. . . protein in the final cell pellet was determined with Bradford reagent (Bio-Rad Laboratories, Richmond, Calif., (12). The microscopic aggregation of ***borrelias*** alone or in the presence of antibodies was graded according to the following scale: 0, single cells with less than. . .

SUMM . . . (10) and Vmp33-specific mAb H4825 (10) have been given. Monoclonal antibody H9724 binds to native and denatured flagellins of different ***Borrelia*** species (9). These antibodies are IgG subclass 2a (IgG2a).

SUMM . . . antibodies were produced for this study. Female, 6-8 week old BALB/c mice (Jackson Laboratory, Bar Harbor, Me.) were used. Freshly-harvested ***borrelia*** were washed with and resuspended in PBS, pH 7.0. The total cellular protein in the suspension was estimated with Bradford. . . the boost. After collection, sera were evaluated by ELISA and GIA. On day 52, the mice received intravenously 2.times.10.sup.8 viable ***borrelias*** in 100 .mu.l of PBS. Fusion of mouse splenocytes with NS1 myeloma cells were performed on day 56 by a. . .

SUMM [0016] The method for ELISA was essentially as described previously (52). For this "dry" ELISA ***borrelias*** at a total protein concentration of 1.4 .mu.g/ml in phosphate-buffered saline (PBS), pH 7.0 were dried onto polystyrene 96-well microtiter plates at 37.degree. C. for 18 h. For a "wet" ELISA ***borrelias*** at a total protein concentration of 3 .mu.g/ml in 15 mM Na.sub.2CO.sub.3-35 mM NaHCO.sub.3 buffer, pH 9.6 were coated onto. . .

SUMM [0018] Indirect immunofluorescence assay (IFA) of fixed, dried cells was performed as described (11, 12). Harvested, fresh ***borrelias*** were washed with RPMI 1640 medium, mixed with a suspension of washed rat erythrocytes in 50% RPMI 1640-50% fetal calf. . .

SUMM . . . antibodies (mAb) to unfixed live spirochetes was assessed by a modification of the procedure of Barbour et al (12). 10.sup.7 ***borrelias*** were washed with 2% (wt/vol) BSA in PBS/Mg (PBS/Mg/BSA) and then resuspended in 0.5 ml of undiluted hybridoma culture supernatant. . .

SUMM . . . mixed together, dialyzed in the dark against PBS for 24 h, and concentrated with a Centriprep-10 (Amicon, Beverly, Mass.). 10.sup.7 ***borrelias*** in log-phase growth were resuspended In RPMI 1640 medium with 10-100 .mu.g/ml of antibody-fluorescein conjugate and examined for fluorescence at. . .

SUMM . . . The growth inhibition assay (GIA) was described previously (53). Briefly, to a 100 .mu.l volume of BSK II containing 2.times.10.sup.6 ***borrelias*** was added an equal volume of heat-inactivated (56.degree. C. for 30 min) mAb or polyclonal antiserum, serially diluted two-fold in BSK II. To evaluate the susceptibility of ***borrelias*** to fresh, nonimmune serum, we applied the same growth inhibition technique using pooled unheated serum from C3H/HeN mice (Taconic, Germantown,. . . immediatelly frozen at -135.degree. C. Heat-inactivated serum from the same mice served as a control. To determine the susceptibility of ***borrelias*** to complement, unheated or heated (56.degree. C. for 30 min) guinea pig complement (Diamedix, Miami, Fla.) was added to each. . .

SUMM . . . electrophoresis (SDS-PAGE) with 15% or 17% acrylamide described previously (2, 11). In some experiments, cleavage of surface-exposed proteins of intact ***borrelias*** with proteinase K (Boehringer-Mannheim) was carried out (51). For this study 490 .mu.l of a suspension containing 5.times.10.sup.8 cells in. . .

SUMM [0026] An assay for adherence of intrinsically-labeled ***borrelias*** to human umbilical vein endothelium (HUVE) cells was carried out essentially as described (62). Briefly, ***borrelias*** were intrinsically radiolabeled with [.sup.35S]-methionine, washed with PBS and resuspended to a density of 1.7.times.10.sup.8 cells per ml in Medium. . . Pharmaceuticals, Irvine, Calif.), and counted by scintillation. The assay was done with triplicate samples and performed twice. Differences between ***borrelia*** populations in adhesion were analyzed by

SUMM [0029] Six-to-eight week old, female C3H/HeN mice (Taconic, Germantown, N.Y.) were used. ***Borrelia*** cells were counted and diluted in BSK II to give the desired inoculum. For live cell immunization, 100 .mu.l of. . . of cultivation; they were scored as negative when no motile spirochetes were seen in forty, 400.times. fields. For evaluation of ***borrelia*** survival in skin, ***borrelias*** were diluted in 1.times.BSK II. The abdominal skin was shaved, and 10.sup.7 ***borrelia*** cells were injected intradermally at 3 or 4 separate locations. Mice were sacrificed at 0.25, 0.5, 2, 6, 9, 12,. . .

SUMM . . . phase. One possible explanation for this is that metabolic activity of the Osp-less mutant was lower than that of wild-type ***borrelias*** . Alternatively, the OspA.sup.-OspB.sup.- mutant may have a slower rate of growth than its parent B311 and, consequently, does not reach the same cell densities as wild-type ***borrelias*** at a particular time point. To examine these possibilities we determined the growth rates of B311 and B313 and measured the amount of ***borrelia*** protein in the final cell pellet.

SUMM [0036] The experiment was performed twice, each time plating in triplicate10.sup.1-10.sup.6 ***borrelias*** per plate. B311 cells grew as colonies with the expected plating efficiency of 50%. The efficiency of B313 plating was. . .

SUMM . . . burgdorferi B311 and B313 cells to HUVE cell monolayers was measured after 4 h at 4.degree. C. At this temperature ***borrelias*** do not detectably enter endothelial cells and ladherence of cells becomes maximal by 4 h (27). The assay was repeated. . .

SUMM . . . nonspecific bactericidal activity of nonimmune serum, in spite of classical and alternative complement pathway activation (38). We asked whether the ***borrelias*** ' ability to resist the nonspecific bactericidal effects of complement might be attributable to Osp

proteins. Accordingly, we first exposed B311. . . was observed at the lowest serum dilution of 1:8. In contrast, the minimum inhibitory titer of nonimmune serum against Osp-less ***borrelias*** was 1:64. In wells with inhibited growth the B313 cells were nonmotile and had large membrane blebs (data not shown).. .' . 4.degree. C.

.sup.cRadioactivity bound to host cells following incubation and washing, expressed as the mean of three samples.

.sup.dDifferences between ***borrelia*** populations in adhesion were analyzed by a Student`s t test (P<0.001).

SUMM [0045] Survival of ***Borrelias*** in Skin

SUMM [0046] In the previous experiment we showed that outer surface lipoproteins might have a role in protecting ***borrelias*** from one nonspecific host-defense, namely, complement. ***Borrelias***. invade the host through the skin, being able to survive in it from a few days to years (59). Accordingly, we next evaluated whether Osp proteins also might protect ***borrelias*** from nonspecific resistance factors in the skin of the mouse, for example, different chemical substances from tissues with antibacterial activity,. . .

SUMM . . . from 18 and 24 h after inoculation was positive. These findings indicated that OspA and/or OspB might not benefit the ***borrelia*** 's survival in the skin. To confirm that cells that survived in the skin retained the same phenotype, 6 randomly chosen. . .

SUMM . . . with live B313 before the spleen fusion. As a screen for surface-directed mABs, we used an ELISA in which whole ***borrelias*** were not dried in the microtiter plate wells. To further evaluate mAbs for surface binding all hybridoma supernatants identified by. . .

SUMM . . . next determined if mAbs 15G6 or 7D4 mAbs recognized similar or identical proteins in other genomic species of Lyme disease ***borrelias*** . The results with 15G6 are shown in FIG. 3; the same results were obtained with 7D4. Representatives of B. afzelii. . .

SUMM . . . to 15G6 mAb by the Western Blot in the wholecell lysates, it was not recognized in the dried and fixed ***borrelias*** .

SUMM [0067] We then assessed the binding of fluorescein-labeled antibodies to fixed and unfixed ***borrelias*** . B313 cells were examined at 3, 15, 30, 60, and 360 min after addition of the 15Gb6 conjugate. The cells. . .

SUMM . . . lacking OspA, B, C, and D was characterized with respect to biological functions and its surface antigens, in particular a ***13*** ***kDa*** protein. Although we focused on a single mutant of B. burgdorferi the results are likely also applicable to other strains of B. burgdorferi sensu lato and the other genomic species of Lyme disease agents. Other isolates of Lyme disease ***borrelias*** have one or more of the Osp proteins (reviewed in ref. 6). The study showed that the Osp-less mutant differed. . .

SUMM . . . B. burgdorferi sensu lato also have a poor plating efficiency on solid medium (47). The diminished ability of aggregated Osp-less ***borrelias*** to move abut the broth medium may explain their slower growth under that condition, but why B313 cells could not. . .

SUMM . . . adhere to human endothelial cells. This indicates that the phenomenon of self-aggregation is not equivalent to the association of the ***borrelias*** with mammalian cells. Prior studies had revealed functions for OspA in endothelial cell adherence and for OspB in cell penetration. . . The findings of the present study are also consistent with a role for OspA and/or OspB in the association of ***borrelias*** with mammalian cells.

SUMM . . . is known about what conferes "serum-resistance" to gram-negative and -positive bacteria; less is known about this aspect of spirochetes. Although ***borrelias*** have two membranes sandwiching a peptidoglycan layer, as do gram-negative bacteria, the outer membrane of ***borrelias*** appears to be more fluid than that of gram-negative bacteria (8) and lack lipid A-containing glycolipids (61). Thus, it was. . . suggest that OspA andfor OspB protect the cells from complement attack. When OspA, B, C, and D are lacking, the ***borrelias*** were more susceptible than OspA.sup.+B.sup.+ cells to unheated, nonimmune serum and to guinea pig complement.

SUMM [0078] Whatever protection OspA and OspB appeared to confer to the ***borrelias*** in serum did not seem to provide an advantage to cells in skin. In this experiment we used two isolates. . .

SUMM . . . B311 and B313 with respect to skin survival, one might expect that the immune responses to intradermal inoculation of viable ***borrelias*** would be comparable. Although the Osp-less mutant lacked two proteins, OspA and OspB, that are immunodominant when syringe inocula of. . .

SUMM . . . which the antibodies produced smaller aggregates and minimal evidence of lysis. The first antibodies were found to bind to a ***13*** ***kDa*** (p13) protein in Western blots. The second group of antibodies did not bind to any component in blots. For the. .

.

SUMM [0082] The evidence that the ***13*** ***kDa*** protein was surface-exposed in the Osp-less mutant was the following: (i) agglutination of viable cells by antibody; (ii) growth inhibition. . .

SUMM . . . of a 14 kDa protein of B. burgdorferi (55). This was identified with a mAb and by immunofluorescence of live ***borrelias*** . In contrast with what was observed by us with mAbs to p13 and by Katona et al. with antibody to. . .

SUMM [0084] The effect of 15G6 on susceptible ***borrelias*** was similar to what was observed with the anti-OspB mAb H6831 (50). Binding to the cells was detectable by direct. . .

SUMM [0086] The results also lead to other questions about the interaction of antibodies and ***borrelias*** , in particular those lacking the known Osp proteins. The target or targets for the second class of mAbs remains to. . .

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J Infect Dis 167:165-72

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E2 2 BARBOUR ALAN D/AU
E3 217 --> BARBOUR ALAN G/AU
E4 18 BARBOUR ALAN GEORGE/AU
E5 3 BARBOUR ALEDIR P/AU
E6 1 BARBOUR ALEXANDER D/AU
E7 1 BARBOUR ALFRED R/AU
E8 2 BARBOUR ANDREW/AU
E9 5 BARBOUR ANDREW D/AU
E10 4 BARBOUR ANDREW P/AU
E11 1 BARBOUR ANDREW PAUL/AU
E12 4 BARBOUR ANGELA H/AU

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YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:570667 BIOSIS
DN PREV200100570667
TI Methods and compositions including a 13kD B. burgdorferi protein.
AU Sadziene, Ariadna; ***Barbour, Alan G.***
ASSIGNEE: Board of Regents, The University of Texas System
PI US 6300101 October 09, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 9, 2001) Vol. 1251, No. 2, pp. No Pagination. e-file. ISSN: 0098-1133.
DT Patent
LA English
AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10-6 to 10-5. One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA+ B+ cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 mug/ml. Class B mAbs did not bind by Western Blot to B. burgdorferi cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistance, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.
AU Sadziene, Ariadna; ***Barbour, Alan G.***
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IT Major Concepts
Human Medicine (Medical Sciences); Methods and Techniques
IT Chemicals & Biochemicals
13 ***kiloDalton*** ***Borrelia*** burgdorferi sensu lato protein
IT Methods & Equipment
13 ***kiloDalton*** ***Borrelia*** burgdorferi sensu

lato protein production method: production method

L6 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:538523 BIOSIS
DN PREV200100538523
TI Methods and compositions including a 13kDa B. burgdorferi protein.
AU Sadziene, Ariadna (1); ***Barbour, Alan G.***
CS (1) San Antonio, TX USA
ASSIGNEE: Board of Regents, The University of Texas System
PI US 6296849 October 02, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 2, 2001) Vol. 1251, No. 1, pp. No Pagination. e-file. ISSN: 0098-1133.
DT Patent
LA English
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AU Sadziene, Ariadna (1); ***Barbour, Alan G.***
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IT Major Concepts
Biochemistry and Molecular Biophysics; Infection
IT Chemicals & Biochemicals
13kDa ***Borrelia*** burgdorferi protein; monoclonal antibodies; outer surface proteins: functional aspects
ORGN Super Taxa
Spirochaetaceae: Spirochaetales, Spirochetes, Eubacteria, Bacteria,

Microorganisms
ORGN Organism Name
Borrelia burgdorferi (Spirochaetaceae): pathogen
ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

L6 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1995:220154 BIOSIS
DN PREV199598234454
TI ***Borrelia*** burgdorferi mutant lacking Osp: Biological and immunological characterization.
AU Sadziene, Ariadna; Thomas, D. Denee; ***Barbour, Alan G. (1)***
CS (1) Dep. Med., Univ. Texas Health Sci. Cent. at San Antonio, San Antonio, TX 78284 USA
SO Infection and Immunity, (1995) Vol. 63, No. 4, pp. 1573-1580. ISSN: 0019-9567.
DT Article
LA English
AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osps). Mutants of B. burgdorferi lacking Osps were selected with polyclonal or monoclonal antibodies at a frequency of 10-6 to 10-5. One mutant that lacked OspA, -B, -C, and -D was further characterized. It was distinguished from the OspA+B+ cells by its (i) autoaggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum and complement sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in mouse skin for the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two types of monoclonal antibodies (MAbs) with growth-inhibiting properties for mutant cells were identified. The first type bound to a ***13*** - ***kDa*** surface protein of B. burgdorferi sensu stricto and of B. afzelii. The MIC of the Fab fragment of one MAb of this type was 0.2 mu-g/ml. The second type of MAb to the Osp-less mutant did not bind to B. burgdorferi components by Western blotting (immunoblotting) but did not bind to unfixed, viable cells in immunofluorescence and growth inhibition assays. These studies revealed possible functions Osp proteins in ***borrelias*** , specifically serum resistance, and indicated that in the absence of Osp proteins, other antigens are expressed or become accessible at the cell surface.
TI ***Borrelia*** burgdorferi mutant lacking Osp: Biological and immunological characterization.
AU Sadziene, Ariadna; Thomas, D. Denee; ***Barbour, Alan G. (1)***
AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osps). Mutants. . . Two types of monoclonal antibodies (MAbs) with growth-inhibiting properties for mutant cells were identified. The first type bound to a ***13*** - ***kDa*** surface protein of B. burgdorferi sensu stricto and of B. afzelii. The MIC of the Fab fragment of one MAb. . . not bind to unfixed, viable cells in immunofluorescence and growth inhibition assays. These studies revealed possible functions Osp proteins in ***borrelias*** , specifically serum resistance, and

indicated that in the absence of Osp proteins, other antigens are expressed or become accessible at. . .
ORGN . . .
Mammalia, Vertebrata, Chordata, Animalia; Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Spirochaetaceae: Eubacteria, Bacteria
ORGN Organism Name
human (Hominidae); mouse (Muridae); ***Borrelia*** burgdorferi (Spirochaetaceae)
ORGN Organism Superterms
animals; bacteria; chordates; eubacteria; humans; mammals; microorganisms; nonhuman mammals; nonhuman vertebrates; primates; rodents; vertebrates

L6 ANSWER 4 OF 4 USPATFULL
AN 2002:140866 USPATFULL
TI Methods and compositions including a 13 kD B. burgdorferi protein
IN Sadziene, Ariadna, Nutley, NJ, UNITED STATES
Barbour, Alan G. , Irvine, CA, UNITED STATES
PI US 2002071847 A1 20020613
AI US 2001-973406 A1 20011009 (9)
RLI Division of Ser. No. US 1994-264036, filed on 22 Jun 1994, PATENTED
Continuation-in-part of Ser. No. US 1993-79601, filed on 22 Jun 1993, PATENTED Continuation of Ser. No. US 1992-924798, filed on 6 Aug 1992, ABANDONED Continuation of Ser. No. US 1989-422881, filed on 18 Oct 1989, ABANDONED
PRAI DK 1988-5902 19881024
DT Utility
FS APPLICATION
LREP FROMMER LAWRENCE & HAUG, 745 FIFTH AVENUE- 10TH FL., NEW YORK, NY, 10151
CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN 5 Drawing Page(s)
LN.CNT 1349
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10.sup.-6 to 10.sup.-5. One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA.sup.+B.sup.+ cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 .mu.g/ml. Class B mAbs did not bind by Western Blot to B. burgdorferi

cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistance, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.

IN ***Barbour, Alan G.*** , Irvine, CA, UNITED STATES

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants. . . and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of. . .

SUMM . . . of basic information about all spirochetes. The spirochete cell is unique in several aspects (33). One of the features of ***borrelia*** is the abundance of one or several lipoproteins in the outer cell membrane (16, 19, 20, 34, 43). Much has. . . been learned about immunogenicity, as well as biochemical and genetic aspects, of these lipoproteins in Lyme disease and relapsing fever ***borrelias*** (4, 16, 19, 35, 37, 42, 64).

SUMM [0004] The lipoproteins OspA and OspB are major contributors to antigenic distinctness of Lyme disease ***borrelias*** (6). Both OspA and OspB are co-transcribed from a single operon located on linear plasmid of 49 kb in B.. . .

SUMM . . . The findings of Cadavid et al indicated that differences in invasive properties and tissues tropism between serotypes of related spirochete ***Borrelia*** turicatae, a relapsing fever agent, may be determined by the expression of a single surface protein that is analogous to. . .

SUMM . . . Osp proteins (51, 54). Our first intent was to characterize morphology and function of the Osp-less mutant. We asked whether ***borrelias*** lacking OspA, B, C, and D would be altered in such functional properties, as (i) generation time, (ii) ability to. . . potential to evoke immune response after intradermal live cell inoculation, and (vi) ability to survive in the skin. Among pathogenic ***borrelias*** the role of surface lipoproteins in these respects have not yet been reported.

SUMM . . . showed the presence of a major low-molecular-weight lipoprotein specific for B. burgdorferi and raised the possibility that it was a ***borrelial*** equivalent of Braun's lipoprotein (36). Another study reported an immunogenic 14 kDa surface protein of B. burgdorferi recognized by sera. . .

SUMM . . . and B. garinii strain Ip90 (1, 17). B. hermsii HS1 serotype 33 (ATCC 35209; ref. 11) was abbreviated to Bh33. ***Borrelias*** were grown in BSK II medium and harvested by methods described previously (3, 5). When culturing tissues from animals, rifampicin. . . (25 .mu.g/ml) were added to the medium. Cells were counted in a Petroff-Hauser chamber by phase-contrast microscopy. In some experiments ***borrelias*** were also grown on solid BSK II medium as described (32, 51). To estimate growth rate, ***borrelias*** at an initial concentration of 2.times.10.sup.6 cells/ml, were grown in tightly capped, 13.times.100-mm polystyrene culture tubes (Falcon Labware, Lincoln Park,. . . protein in the final cell pellet was determined with Bradford reagent (Bio-Rad Laboratories, Richmond, Calif., (12). The microscopic aggregation of ***borrelias*** alone or in the presence

of antibodies was graded according to the following scale: 0, single cells with less than. . .

SUMM . . . (10) and Vmp33-specific mAb H4825 (10) have been given. Monoclonal antibody H9724 binds to native and denatured flagellins of different ***Borrelia*** species (9). These antibodies are IgG subclass 2a (IgG2a).

SUMM . . . antibodies were produced for this study. Female, 6-8 week old BALB/c mice (Jackson Laboratory, Bar Harbor, Me.) were used. Freshly-harvested ***borrelia*** were washed with and resuspended in PBS, pH 7.0. The total cellular protein in the suspension was estimated with Bradford. . . the boost. After collection, sera were evaluated by ELISA and GIA. On day 52, the mice received intravenously 2.times.10.sup.8 viable ***borrelias*** in 100 .mu.l of PBS. Fusion of mouse splenocytes with NS1 myeloma cells were performed on day 56 by a. . .

SUMM [0016] The method for ELISA was essentially as described previously (52). For this "dry" ELISA ***borrelias*** at a total protein concentration of 1.4 .mu.g/ml in phosphate-buffered saline (PBS), pH 7.0 were dried onto polystyrene 96-well microtiter plates at 37.degree. C. for 18 h. For a "wet" ELISA ***borrelias*** at a total protein concentration of 3 .mu.g/ml in 15 mM Na.sub.2CO.sub.3-35 mM NaHCO.sub.3 buffer, pH 9.6 were coated onto. . .

SUMM [0018] Indirect immunofluorescence assay (IFA) of fixed, dried cells was performed as described (11, 12). Harvested, fresh ***borrelias*** were washed with RPMI 1640 medium, mixed with a suspension of washed rat erythrocytes in 50% RPMI 1640-50% fetal calf. . .

SUMM . . . antibodies (mAb) to unfixed live spirochetes was assessed by a modification of the procedure of Barbour et al (12). 10.sup.7 ***borrelias*** were washed with 2% (wt/vol) BSA in PBS/Mg (PBS/Mg/BSA) and then resuspended in 0.5 ml of undiluted hybridoma culture supernatant. . .

SUMM . . . mixed together, dialyzed in the dark against PBS for 24 h, and concentrated with a Centriprep-10 (Amicon, Beverly, Mass.). 10.sup.7 ***borrelias*** in log-phase growth were resuspended In RPMI 1640 medium with 10-100 .mu.g/ml of antibody-fluorescein conjugate and examined for fluorescence at. . .

SUMM . . . The growth inhibition assay (GIA) was described previously (53). Briefly, to a 100 .mu.l volume of BSK II containing 2.times.10.sup.6 ***borrelias*** was added an equal volume of heat-inactivated (56.degree. C. for 30 min) mAb or polyclonal antiserum, serially diluted two-fold in BSK II. To evaluate the susceptibility of ***borrelias*** to fresh, nonimmune serum, we applied the same growth inhibition technique using pooled unheated serum from C3H/HeN mice (Taconic, Germantown,. . . immediatelly frozen at -135.degree. C. Heat-inactivated serum from the same mice served as a control. To determine the susceptibility of ***borrelias*** to complement, unheated or heated (56.degree. C. for 30 min) guinea pig complement (Diamedix, Miami, Fla.) was added to each. . .

SUMM . . . electrophoresis (SDS-PAGE) with 15% or 17% acrylamide described previously (2, 11). In some experiments, cleavage of surface-exposed proteins of intact ***borrelias*** with proteinase K (Boehringer-Mannheim) was carried out (51). For this study 490 .mu.l of a suspension containing 5.times.10.sup.8 cells in. . .

SUMM [0026] An assay for adherence of intrinsically-labeled ***borrelias*** to human umbilical vein endothelium (HUVE) cells was carried out

essentially as described (62). Briefly, ***borrelias*** were intrinsically radiolabeled with [.sup.35S]-methionine, washed with PBS and resuspended to a density of 1.7.times.10.sup.8 cells per ml in Medium. . . Pharmaceuticals, Irvine, Calif.), and counted by scintillation. The assay was done with triplicate samples and performed twice. Differences between ***borrelia*** populations in adhesion were analyzed by

SUMM [0029] Six-to-eight week old, female C3H/HeN mice (Taconic, Germantown, N.Y.) were used. ***Borrelia*** cells were counted and diluted in BSK II to give the desired inoculum. For live cell immunization, 100 .mu.l of. . . of cultivation; they were scored as negative when no motile spirochetes were seen in forty, 400.times. fields. For evaluation of ***borrelia*** survival in skin, ***borrelias*** were diluted in 1.times.BSK II. The abdominal skin was shaved, and 10.sup.7 ***borrelia*** cells were injected intradermally at 3 or 4 separate locations. Mice were sacrificed at 0.25, 0.5, 2, 6, 9, 12,. . .

SUMM . . . phase. One possible explanation for this is that metabolic activity of the Osp-less mutant was lower than that of wild-type ***borrelias*** . Alternatively, the OspA.sup.-OspB.sup.- mutant may have a slower rate of growth than its parent B311 and, consequently, does not reach the same cell densities as wild-type ***borrelias*** at a particular time point. To examine these possibilities we determined the growth rates of B311 and B313 and measured the amount of ***borrelia*** protein in the final cell pellet.

SUMM [0036] The experiment was performed twice, each time plating in triplicate10.sup.1-10.sup.6 ***borrelias*** per plate. B311 cells grew as colonies with the expected plating efficiency of 50%. The efficiency of B313 plating was. . .

SUMM . . . burgdorferi B311 and B313 cells to HUVE cell monolayers was measured after 4 h at 4.degree. C. At this temperature ***borrelias*** do not detectably enter endothelial cells and ladherence of cells becomes maximal by 4 h (27). The assay was repeated. . .

SUMM . . . nonspecific bactericidal activity of nonimmune serum, in spite of classical and alternative complement pathway activation (38). We asked whether the ***borrelias*** ' ability to resist the nonspecific bactericidal effects of complement might be attributable to Osp proteins. Accordingly, we first exposed B311.' . . was observed at the lowest serum dilution of 1:8. In contrast, the minimum inhibitory titer of nonimmune serum against Osp-less ***borrelias*** was 1:64. In wells with inhibited growth the B313 cells were nonmotile and had large membrane blebs (data not shown).. . . 4.degree. C.

.sup.cRadioactivity bound to host cells following incubation and washing, expressed as the mean of three samples.

.sup.dDifferences between ***borrelia*** populations in adhesion were analyzed by a Student`s t test (P<0.001).

SUMM [0045] Survival of ***Borrelias*** in Skin

SUMM [0046] In the previous experiment we showed that outer surface lipoproteins might have a role in protecting ***borrelias*** from one nonspecific host-defense, namely, complement. ***Borrelias*** invade the host through the skin, being able to survive in it from a few days to years (59). Accordingly, we next evaluated whether Osp proteins also might protect ***borrelias*** from nonspecific resistance factors in the skin of the mouse, for example, different chemical substances from tissues with antibacterial activity,. . .

SUMM . . . from 18 and 24 h after inoculation was positive. These findings

indicated that OspA and/or OspB might not benefit the ***borrelia*** 's survival in the skin. To confirm that cells that survived in the skin retained the same phenotype, 6 randomly chosen. . .

SUMM . . . with live B313 before the spleen fusion. As a screen for surface-directed mABs, we used an ELISA in which whole ***borrelias*** were not dried in the microtiter plate wells. To further evaluate mAbs for surface binding all hybridoma supernatants identified by. . .

SUMM . . . next determined if mAbs 15G6 or 7D4 mAbs recognized similar or identical proteins in other genomic species of Lyme disease ***borrelias*** . The results with 15G6 are shown in FIG. 3; the same results were obtained with 7D4. Representatives of B. afzelii. . .

SUMM . . . to 15G6 mAb by the Western Blot in the wholecell lysates, it was not recognized in the dried and fixed ***borrelias*** .

SUMM [0067] We then assessed the binding of fluorescein-labeled antibodies to fixed and unfixed ***borrelias*** . B313 cells were examined at 3, 15, 30, 60, and 360 min after addition of the 15Gb6 conjugate. The cells. . .

SUMM . . . lacking OspA, B, C, and D was characterized with respect to biological functions and its surface antigens, in particular a ***13*** ***kDa*** protein. Although we focused on a single mutant of B. burgdorferi the results are likely also applicable to other strains of B. burgdorferi sensu lato and the other genomic species of Lyme disease agents. Other isolates of Lyme disease ***borrelias*** have one or more of the Osp proteins (reviewed in ref. 6). The study showed that the Osp-less mutant differed. . .

SUMM . . . B. burgdorferi sensu lato also have a poor plating efficiency on solid medium (47). The diminished ability of aggregated Osp-less ***borrelias*** to move abut the broth medium may explain their slower growth under that condition, but why B313 cells could not. . .

SUMM . . . adhere to human endothelial cells. This indicates that the phenomenon of self-aggregation is not equivalent to the association of the ***borrelias*** with mammalian cells. Prior studies had revealed functions for OspA in endothelial cell adherence and for OspB in cell penetration. . . The findings of the present study are also consistent with a role for OspA and/or OspB in the association of ***borrelias*** with mammalian cells.

SUMM . . . is known about what conferes "serum-resistance" to gram-negative and -positive bacteria; less is known about this aspect of spirochetes. Although ***borrelias*** have two membranes sandwiching a peptidoglycan layer, as do gram-negative bacteria, the outer membrane of ***borrelias*** appears to be more fluid than that of gram-negative bacteria (8) and lack lipid A-containing glycolipids (61). Thus, it was. . . suggest that OspA andfor OspB protect the cells from complement attack. When OspA, B, C, and D are lacking, the ***borrelias*** were more susceptible than OspA.sup.+B.sup.+ cells to unheated, nonimmune serum and to guinea pig complement.

SUMM [0078] Whatever protection OspA and OspB appeared to confer to the ***borrelias*** in serum did not seem to provide an advantage to cells in skin. In this experiment we used two isolates. . .

SUMM . . . B311 and B313 with respect to skin survival, one might expect that the immune responses to intradermal inoculation of viable ***borrelias*** would be comparable. Although the Osp-less mutant lacked two proteins, OspA and OspB, that are immunodominant when syringe inocula of. . .

SUMM . . . which the antibodies produced smaller aggregates and minimal

evidence of lysis. The first antibodies were found to bind to a ***13*** ***kDa*** (p13) protein in Western blots. The second group of antibodies did not bind to any component in blots. For the. .

SUMM [0082] The evidence that the ***13*** ***kDa*** protein was surface-exposed in the Osp-less mutant was the following: (i) agglutination of viable cells by antibody; (ii) growth inhibition. . .

SUMM . . . of a 14 kDa protein of B. burgdorferi (55). This was identified with a mAb and by immunofluorescence of live ***borrelias*** . In contrast with what was observed by us with mAbs to p13 and by Katona et al. with antibody to. . .

SUMM [0084] The effect of 15G6 on susceptible ***borrelias*** was similar to what was observed with the anti-OspB mAb H6831 (50). Binding to the cells was detectable by direct. . .

SUMM [0086] The results also lead to other questions about the interaction of antibodies and ***borrelias*** , in particular those lacking the known Osp proteins. The target or targets for the second class of mAbs remains to. . .

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=> s borrel? and ((13 kilodalton?)or(13 kda)or(13,000 dalton?))
L7 45 BORREL? AND ((13 KILODALTON?) OR(13 KDA) OR(13,000 DALTON?))

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 19 DUP REM L7 (26 DUPLICATES REMOVED)

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 19 ANSWERS - CONTINUE? Y/(N):y

L8 ANSWER 1 OF 19 USPATFULL
AN 2003:99695 USPATFULL
TI Use of streptococcus pneumoniae acyl carrier protein synthase crystal structure in diagnostics, antimicrobial drug design, and biosensors
IN Chirgadze, Nicholas Yuri, Indianapolis, IN, UNITED STATES
Briggs, Stephen Lyle, Indianapolis, IN, UNITED STATES
Zhao, Genshi, Indianapolis, IN, UNITED STATES
McAllister, Kelly Ann, Indianapolis, IN, UNITED STATES
PI US 2003068802 A1 20030410
AI US 2001-897645 A1 20010629 (9)
PRAI US 2000-215577P 20000630 (60)
DT Utility
FS APPLICATION
LREP ELI LILLY AND COMPANY, PATENT DIVISION, P.O. BOX 6288, INDIANAPOLIS, IN, 46206-6288
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 9 Drawing Page(s)
LN.CNT 14574
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Provided are methods of purifying and crystallizing Streptococcus pneumoniae acyl carrier protein synthase (AcpS) enzyme, crystals of AcpS, the use of such crystals to determine the three-dimensional structure of AcpS enzymes, and the three-dimensional structure of AcpS. The three-dimensional crystal structure of AcpS can be used in medical diagnostics to produce antibodies that permit detection of Streptococcus pneumoniae both in vitro and in vivo. The three-dimensional crystal structure of AcpS can also be used in pharmaceutical discovery and development to identify and design compounds that inhibit the biochemical activity of AcpS enzyme in bacteria. Inhibitory compounds identified in this way can be optimized by structure/activity studies to

develop antibacterial pharmaceutical compounds useful for the prevention or treatment of bacterial infections.

DETD . . . families and fungi such as: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, ***Borrelia*** (e.g., ***Borrelia*** burgdorferi), Brucellosis, Candidiasis, Campylobacter,Coccidioidomycosis , Cryptococcosis, Dermiatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g.,. . .

DETD . . . As shown in FIG. 1, AcpS is highly expressed in E. coli, and exhibits the predicted molecular weight of approximately ***13*** , ***000*** ***daltons*** . The overexpressed AcpS is purified to apparent homogeneity in two steps using Source S-cation-exchange and gel filtration column chromatography (FIG.. . .

L8 ANSWER 2 OF 19 USPATFULL
AN 2003:93566 USPATFULL
TI Matrix protein compositions for treating infection
IN Gestrelius, Stina, Lund, SWEDEN
Hammarstrom, Lars, Djursholm, SWEDEN
Lyngstadaas, Petter, Nesoddtangen, NORWAY
Andersson, Christer, Vellinge, SWEDEN
Slaby, Ivan, Malno, SWEDEN
Hammargren, Tomas, Malmo, SWEDEN
PA Biora BioEx AB (non-U.S. corporation)
PI US 2003064927 A1 20030403
AI US 2002-156300 A1 20020528 (10)
RLI Division of Ser. No. US 1999-258613, filed on 26 Feb 1999, PENDING
PRAI DK 1998-270 19980227
DK 1998-1328 19981016
US 1998-81551P 19980413 (60)
DT Utility
FS APPLICATION
LREP EDWARDS & ANGELL, LLP., P.O. BOX 9169, BOSTON, MA, 02209
CLMN Number of Claims: 53
ECL Exemplary Claim: 1
DRWN 12 Drawing Page(s)
LN.CNT 2758
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Active enamel substances may be used for the preparation of a pharmaceutical or cosmetic composition for healing of a wound, improving healing of a wound, soft tissue regeneration or repair, or for preventing or treating infection or inflammation.

SUMM . . . enamel matrix proteins may also be used for the treatment of an infection caused by a spirochete such as, e.g., ***Borrelia*** , Leptospira, Treponema or Pseudomonas.

DETD . . . + + + + + +
+ + + + +

EMD fraction A: mostly amelogenin .sup.-26-20 kDa,
EMD fraction B: .sup.-17- ***13*** ***kDa*** proteins,
EMD fraction C: .sup.-10-5 kDa peptides
EMD fraction H: all proteins in EMD above 27 kDa in molecular weight.. . .

L8 ANSWER 3 OF 19 USPATFULL
AN 2002:338194 USPATFULL
TI Oligomeric chaperone proteins
IN Hill, Fergal Conan, Les Martres de Veyre, FRANCE
Chatellier, Jean, Les Martres de Veyre, FRANCE
Fersht, Alan Roy, Cambridge, UNITED KINGDOM
PI US 2002193564 A1 20021219
AI US 2001-7314 A1 20011108 (10)
RLI Continuation-in-part of Ser. No. WO 2000-GB1822, filed on 12 May 2000, UNKNOWN
PRAI GB 1999-11298 19990514
GB 1999-30530 19991223
DT Utility
FS APPLICATION
LREP FROMMER LAWRENCE & HAUG, 745 FIFTH AVENUE- 10TH FL., NEW YORK, NY, 10151
CLMN Number of Claims: 34
ECL Exemplary Claim: 1
DRWN 10 Drawing Page(s)
LN.CNT 2196
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to a polypeptide monomer capable of oligomerisation, said monomer comprising a polypeptide which potentiates protein folding inserted into the sequence of a subunit of an oligomerisable protein scaffold.
DETD . . . Allochromatium vinosum; Amoeba proteus symbiotic bacterium; Aquifex aeolicus; Arabidopsis thaliana; Bacillus sp; Bacillus stearothermophilus; Bacillus subtilis; Bartonella henselae; Bordetella pertussis; ***Borrelia*** burgdorferi; Brucella abortus; Buchnera aphidicola; Burkholderia cepacia; Burkholderia vietnamiensis; Campylobacter jejuni; Caulobacter crescentus; Chiamydia muridarum; Chlamydia trachomatis; Chlamydophila pneumoniae; Clostridium. . .
DETD . . . standards from Pharmacia Biotech. (thyroglobulin, MW=669 kDa; ferritin, MW-440 kDa; aldolase, MW=158 kDa; ovalbulmin, MW=45 kDa; chymotrypsinogen MW=25 kDa; RNase, MW= ***13*** ***kDa***). Molecular weights were determined by logarithmic interpolation.

L8 ANSWER 4 OF 19 USPATFULL
AN 2002:315069 USPATFULL
TI Compositions and methods for treatment of neoplastic disease
IN Terman, David S., Pebble Beach, CA, UNITED STATES
PI US 2002177551 A1 20021128
AI US 2001-870759 A1 20010530 (9)
PRAI US 2000-208128P 20000531 (60)
DT Utility
FS APPLICATION
LREP David S. Terman, P.O. Box 987, Pebble Beach, CA, 93953
CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN 3 Drawing Page(s)
LN.CNT 17323
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention comprises compositions and methods for treating a tumor or neoplastic disease in a host, The methods employ conjugates comprising superantigen polypeptides, nucleic acids with other structures that preferentially bind to tumor cells and are capable of

inducing apoptosis. Also provided are superantigen-glycolipid conjugates and vesicles that are loaded onto antigen presenting cells to activate both T cells and NKT cells. Cell-based vaccines comprise tumor cells engineered to express a superantigen along with glycolipids products which, when expressed, render the cells capable of eliciting an effective anti-tumor immune response in a mammal into which these cells are introduced. Included among these compositions are tumor cells, hybrid cells of tumor cells and accessory cells, preferably dendritic cells. Also provided are tumoricidal T cells and NKT cells devoid of inhitory receptors or inhibitory signaling motifs which are hyperresponsive to the the above compositions and lipid-based tumor associated antigens that can be administered for adoptive immunotherapy of cancer and infectious diseases.

DETD [0246] ***Borrelia*** burgdorfi is the causative agent of Lyme disease. The osp genes are located at a single genetic locus on a. . .

DETD . . . a single gene and enter the lysosome as a single 65-73-kDa precursor chain that is processed into four similarly sized (.about. ***13*** ***kDa***), fairly homologous polypeptides (SAPs A-D) that are heavily glycosylated and have acidic pI values.

L8 ANSWER 5 OF 19 USPATFULL
AN 2002:301560 USPATFULL
TI MATRIX PROTEIN COMPOSITIONS FOR WOUND HEALING
IN GESTRELIUS, STINA, LUND, SWEDEN
HAMMARSTROM, LARS, DJURSHOLM, SWEDEN
LYNGSTADAAS, PETTER, NESODDTANGEN, NORWAY
ANDERSSON, CHRISTER, VELLINGE, SWEDEN
SLABY, IVAN, MALNO, SWEDEN
HAMMARGREN, TOMAS, MALMO, SWEDEN
PI US 2002169105 A1 20021114
US 6503539 B2 20030107
AI US 1999-258613 A1 19990226 (9)
PRAI DK 1998-270 19980227
DK 1998-1328 19981016
US 1998-81551P 19980413 (60)
DT Utility
FS APPLICATION
LREP PETER F CORLESS ESQ, 130 WATER STREET, BOSTON, MA, 02109
CLMN Number of Claims: 53
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 2677
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Active enamel substances may be used for the preparation of a pharmaceutical or cosmetic composition for healing of a wound, improving healing of a wound, soft tissue regeneration or repair, or for preventing or treating infection or inflammation.
SUMM . . . enamel matrix proteins may also be used for the treatment of an infection caused by a spirochete such as, e.g., ***Borrelia*** , Leptospira, Treponema or Pseudomonas.
DETD . . . + + + + + +
+ + + + +

EMD fraction A: mostly amelogenin "26-20 kDa,
EMD fraction B: "17- ***13*** ***kDa*** proteins,

EMD fraction C: "10-5 kDa peptides
EMD fraction H: all proteins in EMD above 27 kDa in molecular weight.. . .

L8 ANSWER 6 OF 19 USPATFULL
AN 2002:251933 USPATFULL
TI Protein scaffold and its use to multimerise monomeric polypeptides
IN Hill, Fergal Conan, Les Martres de Veyre, FRANCE
Chatellier, Jean, Les Martres de Veyre, FRANCE
Fersht, Alan Roy, Cambridge, UNITED KINGDOM
PI US 2002137891 A1 20020926
AI US 2001-7628 A1 20011108 (10)
RLI Continuation-in-part of Ser. No. WO 2000-GB1815, filed on 5 Dec 2000, UNKNOWN
PRAI GB 1999-11298 19990514
GB 1999-28788 19991203
GB 1999-28831 19991206
DT Utility
FS APPLICATION
LREP FROMMER LAWRENCE & HAUG, 745 FIFTH AVENUE- 10TH FL., NEW YORK, NY, 10151
CLMN Number of Claims: 36
ECL Exemplary Claim: 1
DRWN 12 Drawing Page(s)
LN.CNT 2025
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to polypeptide monomer capable of oligomerisation, said monomer comprising a heterologous amino acid sequence inserted into the sequence of a subunit of an oligomerisable protein scaffold.
DETD . . . Allochromatium vinosum; Amoeba proteus symbiotic bacterium; Aquifex aeolicus; Arabidopsis thaliana; Bacillus sp; Bacillus stearothermophilus; Bacillus subtilis; Bartonella henselae; Bordetella pertussis; ***Borrelia*** burgdorferi; Brucella abortus; Buchnera aphidicola; Burkholderia cepacia; Burkholderia vietnamiensis; Campylobacter jejuni; Caulobacter crescentus; Chlamydia muridarum; Chlamydia trachomatis; ChIamydophila pneumoniae; Clostridium. . .
DETD . . . standards from Pharmacia Biotech. (thyroglobulin, MW=669 kDa; ferritin, MW=440 kDa; aldolase, MW=158 kDa; ovalbulmin, MW=45 kDa; chymotrypsinogen MW=25 kDa; RNase, MW= ***13*** ***kDa***). Molecular weights were determined by logarithmic interpolation.

L8 ANSWER 7 OF 19 USPATFULL
AN 2002:140866 USPATFULL
TI Methods and compositions including a 13 kD B. burgdorferi protein
IN Sadziene, Ariadna, Nutley, NJ, UNITED STATES
Barbour, Alan G., Irvine, CA, UNITED STATES
PI US 2002071847 A1 20020613
AI US 2001-973406 A1 20011009 (9)
RLI Division of Ser. No. US 1994-264036, filed on 22 Jun 1994, PATENTED
Continuation-in-part of Ser. No. US 1993-79601, filed on 22 Jun 1993, PATENTED
Continuation of Ser. No. US 1992-924798, filed on 6 Aug 1992, ABANDONED
Continuation of Ser. No. US 1989-422881, filed on 18 Oct 1989, ABANDONED
PRAI DK 1988-5902 19881024
DT Utility
FS APPLICATION
LREP FROMMER LAWRENCE & HAUG, 745 FIFTH AVENUE- 10TH FL., NEW YORK, NY, 10151

CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN 5 Drawing Page(s)
LN.CNT 1349
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10.sup.-6 to 10.sup.-5. One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA.sup.+B.sup.+ cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 .mu.g/ml. Class B mAbs did not bind by Western Blot to B. burgdorferi cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistance, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants. . . and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of. . .

SUMM . . . of basic information about all spirochetes. The spirochete cell is unique in several aspects (33). One of the features of ***borrelia*** is the abundance of one or several lipoproteins in the outer cell membrane (16, 19, 20, 34, 43). Much has. . . been learned about immunogenicity, as well as biochemical and genetic aspects, of these lipoproteins in Lyme disease and relapsing fever ***borrelias*** (4, 16, 19, 35, 37, 42, 64).

SUMM [0004] The lipoproteins OspA and OspB are major contributors to antigenic distinctness of Lyme disease ***borrelias*** (6). Both OspA and OspB are co-transcribed from a single operon located on linear plasmid of 49 kb in B.. . .

SUMM . . . The findings of Cadavid et al indicated that differences in invasive properties and tissues tropism between serotypes of related spirochete ***Borrelia*** turicatae, a relapsing fever agent, may be determined by the expression of a single surface protein that is analogous to. . .

SUMM . . . Osp proteins (51, 54). Our first intent was to characterize

morphology and function of the Osp-less mutant. We asked whether ***borrelias*** lacking OspA, B, C, and D would be altered in such functional properties, as (i) generation time, (ii) ability to. . . potential to evoke immune response after intradermal live cell inoculation, and (vi) ability to survive in the skin. Among pathogenic ***borrelias*** the role of surface lipoproteins in these respects have not yet been reported.

SUMM . . . showed the presence of a major low-molecular-weight lipoprotein specific for B. burgdorferi and raised the possibility that it was a ***borrelial*** equivalent of Braun's lipoprotein (36). Another study reported an immunogenic 14 kDa surface protein of B. burgdorferi recognized by sera. . .

SUMM . . . and B. garinii strain Ip90 (1, 17). B. hermsii HS1 serotype 33 (ATCC 35209; ref. 11) was abbreviated to Bh33. ***Borrelias*** were grown in BSK II medium and harvested by methods described previously (3, 5). When culturing tissues from animals, rifampicin. . . (25 .mu.g/ml) were added to the medium. Cells were counted in a Petroff-Hauser chamber by phase-contrast microscopy. In some experiments ***borrelias*** were also grown on solid BSK II medium as described (32, 51). To estimate growth rate, ***borrelias*** at an initial concentration of 2.times.10.sup.6 cells/ml, were grown in tightly capped, 13.times.100-mm polystyrene culture tubes (Falcon Labware, Lincoln Park,. . . protein in the final cell pellet was determined with Bradford reagent (Bio-Rad Laboratories, Richmond, Calif., (12). The microscopic aggregation of ***borrelias*** alone or in the presence of antibodies was graded according to the following scale: 0, single cells with less than. . .

SUMM . . . (10) and Vmp33-specific mAb H4825 (10) have been given. Monoclonal antibody H9724 binds to native and denatured flagellins of different ***Borrelia*** species (9). These antibodies are IgG subclass 2a (IgG2a).

SUMM . . . antibodies were produced for this study. Female, 6-8 week old BALB/c mice (Jackson Laboratory, Bar Harbor, Me.) were used. Freshly-harvested ***borrelia*** were washed with and resuspended in PBS, pH 7.0. The total cellular protein in the suspension was estimated with Bradford. . . the boost. After collection, sera were evaluated by ELISA and GIA. On day 52, the mice received intravenously 2.times.10.sup.8 viable ***borrelias*** in 100 .mu.l of PBS. Fusion of mouse splenocytes with NS1 myeloma cells were performed on day 56 by a. . .

SUMM [0016] The method for ELISA was essentially as described previously (52). For this "dry" ELISA ***borrelias*** at a total protein concentration of 1.4 .mu.g/ml in phosphate-buffered saline (PBS), pH 7.0 were dried onto polystyrene 96-well microtiter plates at 37.degree. C. for 18 h. For a "wet" ELISA ***borrelias*** at a total protein concentration of 3 .mu.g/ml in 15 mM Na.sub.2CO.sub.3-35 mM NaHCO.sub.3 buffer, pH 9.6 were coated onto. . .

SUMM [0018] Indirect immunofluorescence assay (IFA) of fixed, dried cells was performed as described (11, 12). Harvested, fresh ***borrelias*** were washed with RPMI 1640 medium, mixed with a suspension of washed rat erythrocytes in 50% RPMI 1640-50% fetal calf. . .

SUMM . . . antibodies (mAb) to unfixed live spirochetes was assessed by a modification of the procedure of Barbour et al (12). 10.sup.7 ***borrelias*** were washed with 2% (wt/vol) BSA in PBS/Mg (PBS/Mg/BSA) and then resuspended in 0.5 ml of undiluted hybridoma

culture supernatant. . .

SUMM . . . mixed together, dialyzed in the dark against PBS for 24 h, and concentrated with a Centriprep-10 (Amicon, Beverly, Mass.). 10.sup.7 ***borrelias*** in log-phase growth were resuspended In RPMI 1640 medium with 10-100 .mu.g/ml of antibody-fluorescein conjugate and examined for fluorescence at. . .

SUMM . . . The growth inhibition assay (GIA) was described previously (53). Briefly, to a 100 .mu.l volume of BSK II containing 2.times.10.sup.6 ***borrelias*** was added an equal volume of heat-inactivated (56.degree. C. for 30 min) mAb or polyclonal antiserum, serially diluted two-fold in BSK II. To evaluate the susceptibility of ***borrelias*** to fresh, nonimmune serum, we applied the same growth inhibition technique using pooled unheated serum from C3H/HeN mice (Taconic, Germantown,. . . immediatelly frozen at -135.degree. C. Heat-inactivated serum from the same mice served as a control. To determine the susceptibility of ***borrelias*** to complement, unheated or heated (56.degree. C. for 30 min) guinea pig complement (Diamedix, Miami, Fla.) was added to each. . .

SUMM . . . electrophoresis (SDS-PAGE) with 15% or 17% acrylamide described previously (2, 11). In some experiments, cleavage of surface-exposed proteins of intact ***borrelias*** with proteinase K (Boehringer-Mannheim) was carried out (51). For this study 490 .mu.l of a suspension containing 5.times.10.sup.8 cells in. . .

SUMM [0026] An assay for adherence of intrinsically-labeled ***borrelias*** to human umbilical vein endothelium (HUVE) cells was carried out essentially as described (62). Briefly, ***borrelias*** were intrinsically radiolabeled with [.sup.35S]-methionine, washed with PBS and resuspended to a density of 1.7.times.10.sup.8 cells per ml in Medium. . . Pharmaceuticals, Irvine, Calif.), and counted by scintillation. The assay was done with triplicate samples and performed twice. Differences between ***borrelia*** populations in adhesion were analyzed by

SUMM [0029] Six-to-eight week old, female C3H/HeN mice (Taconic, Germantown, N.Y.) were used. ***Borrelia*** cells were counted and diluted in BSK II to give the desired inoculum. For live cell immunization, 100 .mu.l of. . . of cultivation; they were scored as negative when no motile spirochetes were seen in forty, 400.times. fields. For evaluation of ***borrelia*** survival in skin, ***borrelias*** were diluted in 1.times.BSK II. The abdominal skin was shaved, and 10.sup.7 ***borrelia*** cells were injected intradermally at 3 or 4 separate locations. Mice were sacrificed at 0.25, 0.5, 2, 6, 9, 12,. . .

SUMM . . . phase. One possible explanation for this is that metabolic activity of the Osp-less mutant was lower than that of wild-type ***borrelias*** . Alternatively, the OspA.sup.-OspB.sup.- mutant may have a slower rate of growth than its parent B311 and, consequently, does not reach the same cell densities as wild-type ***borrelias*** at a particular time point. To examine these possibilities we determined the growth rates of B311 and B313 and measured the amount of ***borrelia*** protein in the final cell pellet.

SUMM [0036] The experiment was performed twice, each time plating in triplicate10.sup.1-10.sup.6 ***borrelias*** per plate. B311 cells grew as colonies with the expected plating efficiency of 50%. The efficiency of B313 plating was. . .

SUMM . . . burgdorferi B311 and B313 cells to HUVE cell monolayers was measured after 4 h at 4.degree. C. At this temperature ***borrelias***

do not detectably enter endothelial cells and ladherence of cells becomes maximal by 4 h (27). The assay was repeated. . .

SUMM . . . nonspecific bactericidal activity of nonimmune serum, in spite of classical and alternative complement pathway activation (38). We asked whether the ***borrelias*** ' ability to resist the nonspecific bactericidal effects of complement might be attributable to Osp proteins. Accordingly, we first exposed B311. . . was observed at the lowest serum dilution of 1:8. In contrast, the minimum inhibitory titer of nonimmune serum against Osp-less ***borrelias*** was 1:64. In wells with inhibited growth the B313 cells were nonmotile and had large membrane blebs (data not shown).. . . 4.degree. C.

.sup.cRadioactivity bound to host cells following incubation and washing, expressed as the mean of three samples.

.sup.dDifferences between ***borrelia*** populations in adhesion were analyzed by a Student`s t test (P<0.001).

SUMM [0045] Survival of ***Borrelias*** in Skin

SUMM [0046] In the previous experiment we showed that outer surface lipoproteins might have a role in protecting ***borrelias*** from one nonspecific host-defense, namely, complement. ***Borrelias*** invade the host through the skin, being able to survive in it from a few days to years (59). Accordingly, we next evaluated whether Osp proteins also might protect ***borrelias*** from nonspecific resistance factors in the skin of the mouse, for example, different chemical substances from tissues with antibacterial activity,. . .

SUMM . . . from 18 and 24 h after inoculation was positive. These findings indicated that OspA and/or OspB might not benefit the ***borrelia*** 's survival in the skin. To confirm that cells that survived in the skin retained the same phenotype, 6 randomly chosen. . .

SUMM . . . with live B313 before the spleen fusion. As a screen for surface-directed mABs, we used an ELISA in which whole ***borrelias*** were not dried in the microtiter plate wells. To further evaluate mAbs for surface binding all hybridoma supernatants identified by. . .

SUMM . . . next determined if mAbs 15G6 or 7D4 mAbs recognized similar or identical proteins in other genomic species of Lyme disease ***borrelias*** . The results with 15G6 are shown in FIG. 3; the same results were obtained with 7D4. Representatives of B. afzelii. . .

SUMM . . . to 15G6 mAb by the Western Blot in the wholecell lysates, it was not recognized in the dried and fixed ***borrelias*** .

SUMM [0067] We then assessed the binding of fluorescein-labeled antibodies to fixed and unfixed ***borrelias*** . B313 cells were examined at 3, 15, 30, 60, and 360 min after addition of the 15Gb6 conjugate. The cells. . .

SUMM . . . lacking OspA, B, C, and D was characterized with respect to biological functions and its surface antigens, in particular a ***13*** ***kDa*** protein. Although we focused on a single mutant of B. burgdorferi the results are likely also applicable to other strains of B. burgdorferi sensu lato and the other genomic species of Lyme disease agents. Other isolates of Lyme disease ***borrelias*** have one or more of the Osp proteins (reviewed in ref. 6). The study showed that the Osp-less mutant differed. . .

SUMM . . . B. burgdorferi sensu lato also have a poor plating efficiency on solid medium (47). The diminished ability of aggregated Osp-less ***borrelias*** to move abut the broth medium may explain their slower growth under that condition, but why B313 cells could not. . .

SUMM . . . adhere to human endothelial cells. This indicates that the

phenomenon of self-aggregation is not equivalent to the association of the ***borrelias*** with mammalian cells. Prior studies had revealed functions for OspA in endothelial cell adherence and for OspB in cell penetration. . . The findings of the present study are also consistent with a role for OspA and/or OspB in the association of ***borrelias*** with mammalian cells.

SUMM . . . is known about what conferes "serum-resistance" to gram-negative and -positive bacteria; less is known about this aspect of spirochetes. Although ***borrelias*** have two membranes sandwiching a peptidoglycan layer, as do gram-negative bacteria, the outer membrane of ***borrelias*** appears to be more fluid than that of gram-negative bacteria (8) and lack lipid A-containing glycolipids (61). Thus, it was. . . suggest that OspA andfor OspB protect the cells from complement attack. When OspA, B, C, and D are lacking, the ***borrelias*** were more susceptible than OspA.sup.+B.sup.+ cells to unheated, nonimmune serum and to guinea pig complement.

SUMM [0078] Whatever protection OspA and OspB appeared to confer to the ***borrelias*** in serum did not seem to provide an advantage to cells in skin. In this experiment we used two isolates. . .

SUMM . . . B311 and B313 with respect to skin survival, one might expect that the immune responses to intradermal inoculation of viable ***borrelias*** would be comparable. Although the Osp-less mutant lacked two proteins, OspA and OspB, that are immunodominant when syringe inocula of. . .

SUMM . . . which the antibodies produced smaller aggregates and minimal evidence of lysis. The first antibodies were found to bind to a ***13*** ***kDa*** (p13) protein in Western blots. The second group of antibodies did not bind to any component in blots. For the. .

SUMM [0082] The evidence that the ***13*** ***kDa*** protein was surface-exposed in the Osp-less mutant was the following: (i) agglutination of viable cells by antibody; (ii) growth inhibition. . .

SUMM . . . of a 14 kDa protein of B. burgdorferi (55). This was identified with a mAb and by immunofluorescence of live ***borrelias*** . In contrast with what was observed by us with mAbs to p13 and by Katona et al. with antibody to. . .

SUMM [0084] The effect of 15G6 on susceptible ***borrelias*** was similar to what was observed with the anti-OspB mAb H6831 (50). Binding to the cells was detectable by direct. . .

SUMM [0086] The results also lead to other questions about the interaction of antibodies and ***borrelias*** , in particular those lacking the known Osp proteins. The target or targets for the second class of mAbs remains to. . .

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L8 ANSWER 8 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2003:560 BIOSIS
DN PREV200300000560
TI Elimination of channel-forming activity by insertional inactivation of the p13 gene in ***Borrelia*** burgdorferi.
AU Ostberg, Yngve; Pinne, Marija; Benz, Roland; Rosa, Patricia; Bergstrom, Sven (1)
CS (1) Department of Molecular Biology, Umea University, SE-901 87, Umea, Sweden: sven.bergstrom@molbiol.umu.se Sweden
SO Journal of Bacteriology, (December 2002, 2002) Vol. 184, No. 24, pp. 6811-6819. print.
ISSN: 0021-9193.
DT Article
LA English
AB P13 is a chromosomally encoded ***13*** - ***kDa*** integral outer membrane protein of the Lyme disease agent, ***Borrelia*** burgdorferi. The aim of this study was to investigate the function of the

P13 protein. Here, we inactivated the p13 gene by targeted mutagenesis and investigated the porin activities of outer membrane proteins by using lipid bilayer experiments. Channel-forming activity was lost in the p13 mutant compared to wild-type B. burgdorferi, indicating that P13 may function as a porin. We purified native P13 to homogeneity by fast performance liquid chromatography and demonstrated that pure P13 has channel-forming activity with a single-channel conductance in 1 M KCl of 3.5 nS, the same as the porin activity that was lost in the p13 mutant. Further characterization of the channel formed by P13 suggested that it is cation selective and voltage independent. In addition, no major physiological effects of the inactivated p13 gene could be detected under normal growth conditions. The inactivation of p13 is the first reported inactivation of a gene encoding an integral outer membrane protein in B. burgdorferi. Here, we describe both genetic and biophysical experiments indicating that P13 in B. burgdorferi is an outer membrane protein with porin activity.

TI Elimination of channel-forming activity by insertional inactivation of the p13 gene in ***Borrelia*** burgdorferi.

AB P13 is a chromosomally encoded ***13*** - ***kDa*** integral outer membrane protein of the Lyme disease agent, ***Borrelia*** burgdorferi. The aim of this study was to investigate the function of the P13 protein. Here, we inactivated the p13. . .

ORGN Super Taxa
Spirochaetaceae: Spirochaetales, Spirochetes, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
Borrelia burgdorferi (Spirochaetaceae): strain-ATCC 35210, strain-B31

ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

GEN ***Borrelia*** burgdorferi p13 gene (Spirochaetaceae): insertional activation

L8 ANSWER 9 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

AN 2001:570667 BIOSIS

DN PREV200100570667

TI Methods and compositions including a 13kD B. burgdorferi protein.

AU Sadziene, Ariadna; Barbour, Alan G.
ASSIGNEE: Board of Regents, The University of Texas System

PI US 6300101 October 09, 2001

SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 9, 2001) Vol. 1251, No. 2, pp. No Pagination. e-file. ISSN: 0098-1133.

DT Patent

LA English

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10-6 to 10-5. One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA+ B+ cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells.

The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 mug/ml. Class B mAbs did not bind by Western Blot to B. burgdorferi cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistance, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants. . . and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of. . .

IT Major Concepts
Human Medicine (Medical Sciences); Methods and Techniques

IT Chemicals & Biochemicals
13 ***kiloDalton*** ***Borrelia*** burgdorferi sensu lato protein

IT Methods & Equipment
13 ***kiloDalton*** ***Borrelia*** burgdorferi sensu lato protein production method: production method

L8 ANSWER 10 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3

AN 2001:538523 BIOSIS

DN PREV200100538523

TI Methods and compositions including a 13kDa B. burgdorferi protein.

AU Sadziene, Ariadna (1); Barbour, Alan G.

CS (1) San Antonio, TX USA
ASSIGNEE: Board of Regents, The University of Texas System

PI US 6296849 October 02, 2001

SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 2, 2001) Vol. 1251, No. 1, pp. No Pagination. e-file.
ISSN: 0098-1133.

DT Patent

LA English

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10-6 to 10-5. One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA+ B+ cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells.

The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 mug/ml. Class B mAbs did not bind by Western blot to B. burgdorferi cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistande, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.

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IT Major Concepts
Biochemistry and Molecular Biophysics; Infection

IT Chemicals & Biochemicals
13kDa ***Borrelia*** burgdorferi protein; monoclonal antibodies; outer surface proteins: functional aspects

ORGN Super Taxa
Spirochaetaceae: Spirochaetales, Spirochetes, Eubacteria, Bacteria; Microorganisms

ORGN Organism Name
Borrelia burgdorferi (Spirochaetaceae): pathogen

ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

L8 ANSWER 11 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4

AN 2001:301202 BIOSIS

DN PREV200100301202

TI P13, an integral membrane protein of ***Borrelia*** burgdorferi, is C-terminally processed and contains surface-exposed domains.

AU Noppa, Laila; Ostberg, Yngve; Lavrinovicha, Marija; Bergstrom, Sven (1)

CS (1) Department of Microbiology, Umea University, SE-901 87, Umea: sven.bergstrom@micro.umu.se Sweden

SO Infection and Immunity, (May, 2001) Vol. 69, No. 5, pp. 3323-3334. print. ISSN: 0019-9567.

DT Article

LA English

SL English

AB To elucidate antigens present on the bacterial surface of ***Borrelia*** burgdorferi sensu lato that may be involved in pathogenesis, we characterized a protein, P13, with an apparent molecular mass of ***13*** ***kDa*** . The protein was immunogenic and was expressed in large amounts during in vitro cultivation compared to other known

antigens. An immunofluorescence assay, immunoelectron microscopy, and protease sensitivity assays indicated that P13 is surface exposed. The deduced sequence of the P13 peptide revealed a possible signal peptidase type I cleavage site, and computer analysis predicted that P13 is an integral membrane protein with three transmembrane-spanning domains. Mass spectrometry, in vitro translation, and N- and C-terminal amino acid sequencing analyses indicated that P13 was posttranslationally processed at both ends and modified by an unknown mechanism. Furthermore, p13 belongs to a gene family with five additional members in B. burgdorferi sensu stricto. The p13 gene is located on the linear chromosome of the bacterium, in contrast to five paralogous genes, which are located on extrachromosomal plasmids. The size of the p13 transcript was consistent with a monocistronic transcript. This new gene family may be involved in functions that are specific for this spirochete and its pathogenesis.

TI P13, an integral membrane protein of ***Borrelia*** burgdorferi, is C-terminally processed and contains surface-exposed domains.

AB To elucidate antigens present on the bacterial surface of ***Borrelia*** burgdorferi sensu lato that may be involved in pathogenesis, we characterized a protein, P13, with an apparent molecular mass of ***13*** ***kDa*** . The protein was immunogenic and was expressed in large amounts during in vitro cultivation compared to other known antigens. An. . .

ORGN Super Taxa
Spirochaetaceae: Spirochaetales, Spirochetes, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
Borrelia burgdorferi (Spirochaetaceae): pathogen

ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

GEN ***Borrelia*** burgdorferi p13 gene (Spirochaetaceae): transcript

L8 ANSWER 12 OF 19 LIFESCI COPYRIGHT 2003 CSA

AN 2002:49123 LIFESCI

TI Methods and compositions including a 13kDa B. burgdorferi protein

AU Sadziene, A.; Barbour, A.G.

CS Board of Regents, The University of Texas System

SO (20011002) . US Patent: 6296849; US CLASS: 424/141.1; 424/1.49; 424/150.1; 424/164.1; 424/184.1; 424/234.1; 435/7.32; 435/69.3; 530/825.

DT Patent

FS W2

LA English

SL English

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of B. burgdorferi lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of 10 super(-6) to 10 super(-5). One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA super(+) B super(+) cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of

the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 mu g/ml. Class B mAbs did not bind by Western blot to B. burgdorferi cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistance, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.

AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants. . . and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to ***13*** ***kDa*** surface proteins of B. burgdorferi sensu stricto and of B. afzelii. The minimum inhibitory concentration of the Fab fragment of. . .

UT Antibodies; Patents; OspA protein; OspB protein; OspC protein; OspD protein; ***Borrelia*** burgdorferi

L8 ANSWER 13 OF 19 USPATFULL

AN 2000:160592 USPATFULL

TI ***Borrelia*** burgdorferi outer membrane proteins

IN Skare, Jonathan T., College Station, TX, United States
Shang, Ellen S., Calabasas, CA, United States
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Blanco, David R., Calabassas, CA, United States
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PA The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

PI US 6153194 20001128

AI US 1998-183217 19981029 (9)

RLI Continuation of Ser. No. US 1997-787367, filed on 21 Jan 1997, now abandoned

PRAI US 1996-10321P 19960122 (60)

DT Utility

FS Granted

EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Ryan, V.

LREP Fulbright & Jaworski L.L.P.

CLMN Number of Claims: 5

ECL Exemplary Claim: 1

DRWN 43 Drawing Figure(s); 24 Drawing Page(s)

LN.CNT 3234

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention presents three B. burgdorferi membrane proteins: Oms28, Oms45, and Oms66, each of about 28, 45, and 66 kDa respectively; and with average single channel conductances of about 0.6, 0.22, and 9.7 nS, respectively. Also disclosed are the methods for purifying these

proteins from B. burgdorferi, methods for producing antibodies to these proteins, and the resulting antibodies. These proteins and their immunogenic fragments, and antibodies capable of binding to them are useful for inducing an immune response to pathogenic B. burgdorferi as well as providing a diagnostic target for Lyme disease. Further disclosed are the nucleotide and amino acid sequences, the cloning of the genes encoding the proteins and their recombinant proteins, and methods for obtaining the foregoing. Other B. burgdorferi outer membrane spanning proteins (Oms) obtainable by the isolation and purification methods of the present invention.

TI ***Borrelia*** burgdorferi outer membrane proteins

SUMM This invention relates generally to membrane proteins and specifically to ***Borrelia*** burgdorferi membrane proteins, particularly outer membrane-spanning porin proteins, which are used to induce a protective immune response in animals. Such. . .

SUMM Lyme disease is a tick-borne infection with worldwide distribution caused by ***Borrelia*** burgdorferi sensu lato. ***Borrelia*** burgdorferi sensu stricto (hereinafter referred to as "B. burgdorferi") initially causes a flu-like systemic illness that, if untreated, may develop. . . 321:586-596 (1989)}. Although the clinical manifestations of Lyme disease have been well documented, basic knowledge relating the pathogenesis of Lyme ***borreliosis*** to specific molecular components, specifically outer membrane (OM) proteins, has been lacking due primarily to the lability of the OM. .

.

SUMM . . . proteins, and the resulting antibodies. These proteins and their immunogenic fragments may function as vaccine candidates to protect against Lyme ***borreliosis*** , and antibodies capable of binding to them may have bactericidal activity against pathogenic B. burgdorferi. These proteins and the antibodies. . .

DETD . . . proteins, and the resulting antibodies. These proteins and their immunogenic fragments may function as vaccine candidates to protect against Lyme ***borreliosis*** , and antibodies capable of binding to them may have bactericidal activity against pathogenic B. burgdorferi. These proteins and the antibodies. . .

DETD Oms28, Oms45, and Oms66 can be identified in different ***Borrelia*** genus (hereinafter referred to as " ***Borrelia*** ") by their immunoreactivity with antibodies raised against the Oms28, Oms45, and Oms66 described in or derived from (e.g. as recombinant. . . antigenic fragments) the "EXAMPLES" section, below. Immunoblot studies may be conducted to determine whether there is a strong correlation between ***Borrelia*** , B. burgdorferi sensu lato, or B. burgdorferi sensu stricto (the latter is hereinafter referred to as B. burgdorferi) pathogenicity and. . . Oms45, and Oms66 (or proteins isolated and purified by the methods of the present invention) are present and specific for ***Borrelia*** , B. burgdorferi sensu lato, or B. burgdorferi. An immunoblot assay may be conducted, for example, similar to that described in. . . burgdorferi isolates. Similarly, hybridization assays may be conducted to determine whether Oms28, Oms45, and Oms66 nucleotide sequences are specific to ***Borrelia*** , B. burgdorferi sensu lato, or B. burgdorferi, under moderate or stringent hybridization assay conditions. The nucleotide and amino acid sequences. . . as that of GenBank) to determine their homology or specificity vis-a-vis other organisms. In the following discussion, for convenience, only ***Borrelia*** is mentioned. However, one skilled in the art

would understand that according to the specificities of the Oms28, Oms45, and Oms66 proteins, antibodies, and nucleotide sequences, they may be used in relation to ***Borrelia*** , ***Borrelia*** sensu lato, and/or B. burgdorferi, in the following discussion, e.g. in regard to diagnostic tests, therapeutics and vaccines. For example,. . . based on the findings of Example 2, below. In the present invention, B. burgdorferi sensu lato refers to species of ***Borrelia*** that cause Lyme disease or diseases resembling Lyme disease; such species include B. garinii, B. afzelii, and B. burgdorferi. In. . .

DETD . . . Preferably, the determinative biological characteristic/activity is the retention of at least one immunoepitope. Preferably, when used in an immunoassay for ***Borrelia*** , these proteins are immunoreactive with antibodies directed to ***Borrelia*** but not immunoreactive with antibodies directed against other antigens not specific to ***Borrelia*** found in a biological sample. More preferably, these proteins are immunoreactive with antibodies specific to ***Borrelia*** sensu lato, and most preferably B. burgdorferi.

DETD . . . from mammals, such as humans, wild or domestic mammals. More preferably, these proteins and the immunoassays can additionally distinguish between ***Borrelia*** and other spirochetes. More preferably, these proteins and immunoassays are specific for ***Borrelia*** sensu lato, and most preferably B. burgdorferi. Alternatively, the fragments of nucleotide sequences can be nucleotide probes of at least 20 nucleotides in length. Preferably, when used in a hybridization assay for ***Borrelia*** , under moderate to stringent hybridization condition, these probes do not detectably hybridize to the nucleotide sequences of non- ***Borrelia*** organisms which are found in a biological sample. More preferably, these hybridization assays are specific for ***Borrelia*** sensu lato, and most preferably B. burgdorferi; particularly under moderate to stringent hybridization condition. For example, when used to assay. . .

DETD . . . at least 8; more preferably, 5 to 6; and most preferably, 4 amino acids. Preferably, the protein is specific to ***Borrelia*** , more preferably ***Borrelia*** sensu lato, and most preferably B. burgdorferi. Alternatively, the protein retains one or more biological functions of ***Borrelia*** , more preferably ***Borrelia*** sensu lato, and most preferably B. burgdorferi.

DETD Preferably, when used in an immunoassay for ***Borrelia*** , these proteins are immunoreactive with antibodies directed to ***Borrelia*** but not detectably immunoreactive with non- ***Borrelia*** specific antibodies found in a biological sample, and other spirochetes. More preferably, these proteins and immunoassays are specific for ***Borrelia*** sensu lato, and most preferably B. burgdorferi.

DETD . . . between pathogenic B. burgdorferi and non-pathogenic B. burgdorferi. Alternatively, or additionally, these proteins retain one or more biological functions of ***Borrelia*** , preferably ***Borrelia*** sensu lato, and most preferably B. burgdorferi. Thus, preferably, the fragment of these proteins claimed in this application contains at least one immunogenic epitope of ***Borrelia*** , preferably ***Borrelia*** sensu lato, and most preferably B. burgdorferi. Another example of a fragment is one which is capable of being bound. . .

DETD . . . and/or humoral response in an animal vaccinated with the proteins. More preferably, the cellular and/or humoral response is directed against ***Borrelia*** , preferably ***Borrelia*** sensu

lato, more preferably B. burgdorferi, and most preferably pathogenic B. burgdorferi. Most preferably, animals vaccinated with these proteins are immunized against disease caused by ***Borrelia*** , preferably ***Borrelia*** sensu lato, or more preferably B. burgdorferi (such as Lyme disease) or such vaccinations ameliorate the disease in infected animals.. . .

DETD . . . of these proteins and their immunogenic epitopes. As described above, preferably, too, each variant retains at least one immunoepitope of ***Borrelia*** , preferably ***Borrelia*** sensu lato, more preferably B. burgdorferi, and most preferably pathogenic B. burgdorferi. Preferably the immunoepitope is specific to ***Borrelia*** , preferably ***Borrelia*** sensu lato, more preferably B. burgdorferi, and most preferably pathogenic B. burgdorferi.

DETD The bacterial genes encoding the Oms28, Oms45, and Oms66 proteins can be derived from any strain ***Borrelia*** . Preferably the genes are from B. burgdorferi.

DETD In one embodiment, the invention provides a pharmaceutical composition useful for inducing an immune response to ***Borrelia*** in an animal comprising an immunologically effective amount of Oms28, Oms45, and/or Oms66 in a pharmaceutically acceptable carrier. The term "immunogenically effective amount," as used in describing the invention, is meant to denote that amount of ***Borrelia*** antigen which is necessary to induce in an animal the production of an immune response to the respective ***Borrelia*** . Oms28, Oms45, and Oms66 are particularly useful in sensitizing the immune system of an animal such that, as one result, an immune response is produced which ameliorates the effect of infection by these ***Borrelia*** . Oms28, Oms45, and Oms66 proteins i.e., their variants, functional equivalents, and derivatives, which are effective as vaccines against the disease caused by ***Borrelia*** antigen, can be screened for using the methods known in the art such as described in Fikrig, E., et al.,. . .

DETD . . . the above compositions, and be administered, e.g. orally. The vaccines can also be added to baits against potential carriers of ***Borrelia*** such as rodents so that they will not be infected by ***Borrelia*** and be carriers in spreading ***Borrelia*** and the disease to humans and other animals, such as domestic animals.

DETD In another embodiment, a method of inducing an immune response to ***Borrelia*** in animals is provided. Many different techniques exist for the timing of the immunizations when a multiple immunization regimen is. . . they will be spaced two to four weeks apart. Suitable subjects include any animal susceptible to infection by the respective ***Borrelia*** . The animals are preferably mammals. Examples of the mammals are: humans, domestic and wild mammals. The domestic mammals include: livestock. . .

DETD . . . antibodies are those large enough to produce the desired effect in which the onset symptoms of the disease caused by ***Borrelia*** are ameliorated. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic. . .

DETD In a further embodiment, the invention provides a method of detecting a pathogenic ***Borrelia*** -associated disorder in a subject comprising contacting a cell component with a reagent which binds to the cell component. The cell. . .

DETD . . . the cell component is nucleic acid, it may be necessary to amplify the nucleic acid prior to binding with a ***Borrelia***

specific probe. Preferably, polymerase chain reaction (PCR) is used, however, other nucleic acid amplification procedures such as ligase chain reaction. . .

DETD . . . antibodies, preferably monoclonal antibodies and SCA, of the invention can also be used to monitor the course of amelioration of ***Borrelia*** associated disorder. Thus, by measuring the increase or decrease of Oms28, Oms45, and/or Oms66 proteins or antibodies to Oms28, Oms45,. . .

DETD . . . Oms28, Oms45, and Oms66 proteins or nucleic acid sequences, in combination with other proteins or nucleic acid sequences specific for ***Borrelia*** . Similarly, the compositions, e.g. for immunoassays or vaccinations, may consist of Oms28, Oms45, or Oms66, singly. Alternatively, they may consist of a cocktail containing both Oms28, Oms45, and Oms66, or these proteins in combination with other proteins specific for ***Borrelia*** . The antibody compositions may consist of antibodies specific to Oms28, Oms45, or Oms66. Alternatively, they may consist of a cocktail containing antibodies to Oms28, Oms45, and Oms66, or to these proteins and other proteins specific for ***Borrelia*** . The hybridization assays are preferably run at moderate to stringent conditions. The immunoassays are preferably conducted under conditions of reduced. . .

DETD In more detail, we have isolated and purified OMV from ***Borrelia*** burgdorferi strain B31 based on methods developed for isolation of T. pallidum OMV. Purified OMV exhibited distinct porin activities with. .

DETD ***Borrelia*** burgdorferi sensu stricto strain B31 was used in the experiments presented in this report and will be referred to as. . .

DETD . . . of TX-114 detergent phase protein from the passage 10 B31 OMV consisted of 30 spots with molecular masses ranging from ***13*** ***kDa*** to 65 kDa (FIG. 3A and summarized in Table I). No differences were detected in the composition of the passage. . .

DETD . . . see Table I). The TX-114 detergent phase protein from the avirulent ATCC B31 OMV consisted of sixteen spots ranging from ***13*** ***kDa*** to 65 kDa, each with molecular masses and isoeletric points indistinguishable from proteins observed in either the passage 10 B31. . . 60:4662-4672 (1992)} suggested that the abundant 31-kDa and 33-kDa proteins were the OspA and OspB lipoproteins, respectively, and that the ***13*** - ***kDa*** species was an uncharacterized lipoprotein. ECL Western blotting with monoclonal antibodies (MAbs) specific for OspA and OspB confirmed that these. . .

DETD . . . avirulent ATCC B31 OMV (FIGS. 6A and 6B respectively) indicated that both preparations contained OspA, OspB, and a previously identified ***13*** - ***kDa*** lipoprotein {Norris, S. J. et al., Infect. Immun., 60:4662-4672 (1992)}. The passage 5 B31 OMV sample had additional lipoproteins with. . .

DETD . . . the passage 10 and passage 48 isolate. Because the passage 48 isolate retains infectivity in the rabbit model of Lyme ***borreliosis*** {Foley, D. M. et al., J. Clin. Invest., 96:965-975 (1995)}, these three OMV proteins, designated 19.5d, 28, and 35a (Table. . .

DETD We have chosen the acronym, "Oms", to designate the outer membrane spanning proteins shared by virulent and avirulent ***Borrelia*** burgdorferi strain B31. We have also chosen the acronym, Oms.sup.vsa, for Oms that are virulent strain-associated, to designate those candidate. . .

DETD This Example describes the FPLC purification of the 0.6 nS native porin protein from ***Borrelia*** burgdorferi that we have designated Oms28 for outer membrane-spanning protein 28. In addition, we have cloned and determined the nucleotide. . .

DETD . . . of B. burgdorferi, the first to be described. As such, it is of potential relevance to the pathogenesis of Lyme ***borreliosis*** , and to the physiology of the spirochete.

DETD ***Borrelia*** burgdorferi sensu stricto strain B31 was used in most of the experiments presented in this study and will be referred. . .

DETD . . . rabbit tissue and cultivated in BSK-II media. European B. burgdorferi low-passage isolates 2872-2, 2872-3, 2872-6, and 3251-5, as well as ***Borrelia*** garinii, were kindly provided by Dr. Vittorio Sambri, University of Bologna, Italy. ***Borrelia*** hermsiiserotype 7 (low-passage isolate) and serotype 33 (high-passage isolate) were both generously provided by Dr. Alan Barbour, University of Texas. . .

DETD Outer Membrane Localization of Oms28 in ***Borrelia*** burgdorferi

DETD . . . or low-passage European isolates (FIG. 16). Additionally, we examined if a protein similar to Oms28 was present in a related ***Borrelia*** , ***Borrelia*** garinii, and in the etiologic agents of relapsing fever and syphilis, ***Borrelia*** hermsii and T. pallidum, respectively (FIG. 16). All American and European isolates tested contained an Oms28-like protein although N40 and. . .

DETD Porin Activity of Oms66 from ***Borrelia*** burgdorferi

DETD Until recently no outer membrane-spanning (Oms) proteins had been described for ***Borrelia*** burdorferi, the etiologic agent of Lyme disease even though Oms proteins had been observed previously by freeze fracture electron microscopy. . .

DETD . . . on the surface of B. burgdorferi it is tempting to speculate that Oms66 may function as an effective target for ***borrelicidal*** antibody binding and, as such, may be an effective vaccine candidate to protect against Lyme ***borreliosis*** .

DETD ---

SEQUENCE LISTING

- <160> NUMBER OF SEQ ID NOS: 9

- <210> SEQ ID NO 1

<211> LENGTH: 1129

<212> TYPE: DNA

<213> ORGANISM: ***Borrelia*** burgdorferi

- <400> SEQUENCE: 1

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60

- tttagttatg ttttaaaata ataaaaataa atgtttaaat aaggagaatt aa -. . .
acatttccaa tatttacttg ttgcatattt at - #atcttaac
1080

1129taagg aaggagaatt aatttttgaa taaagaata

- <210> SEQ ID NO 2

<211> LENGTH: 257

<212> TYPE: PRT

<213> ORGANISM: ***Borrelia*** burgdorferi

- <400> SEQUENCE: 2

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Description of Artificial

- <400> SEQUENCE: 5

31 aaca atgcaaatat t

- <210> SEQ ID NO 6
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: ***Borrelia*** burgdorferi
- <400> SEQUENCE: 6
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15
- <210> SEQ ID NO 7
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: ***Borrelia*** burgdorferi
- <400> SEQUENCE: 7
- Tyr Lys Leu Gly Leu Thr Lys
1 5
- <210> SEQ ID NO 8
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: ***Borrelia*** burgdorferi
- <400> SEQUENCE: 8
- Ile Asn Asp Lys Asn Thr Tyr Leu Ile Leu Gl - #n Met Gly Thr. . . Phe
15
- Gly Ile Asp Pro Phe Ala Ser
20
- <210> SEQ ID NO 9
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: ***Borrelia*** burgdorferi
- <400> SEQUENCE: 9
- Asp Thr Gly Glu Lys Glu Ser Trp Ala Ile Ly - #s
10

CLM What is claimed is:

1. An outer membrane spanning protein isolated from ***Borrelia*** outer membrane comprising Oms28.

5. A method of producing antibodies which recognize ***Borrelia*** burgdorferi comprising administering to a host an immunogenically effective amount of Osm28.

L8 ANSWER 14 OF 19 USPATFULL
AN 2000:9723 USPATFULL
TI Unique nucleotide and amino acid sequence and uses thereof
IN Summers, Max D., Bryan, TX, United States
Braunagel, Sharon C., Bryan, TX, United States
Hong, Tao, Bryan, TX, United States
PA The Texas A & M University System, College Station, TX, United States (U.S. corporation)
PI US 6017734 20000125
AI US 1997-792832 19970130 (8)
RLI Continuation-in-part of Ser. No. US 1996-678435, filed on 3 Jul 1996, now abandoned
PRAI US 1995-955P 19950707 (60)
DT Utility
FS Granted

EXNAM Primary Examiner: Elliott, George C.; Assistant Examiner: Schwartzman, Robert
LREP Arnold, White & Durkee
CLMN Number of Claims: 56
ECL Exemplary Claim: 1
DRWN 47 Drawing Figure(s); 24 Drawing Page(s)
LN.CNT 7846
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Provided are hydrophobic targeting sequences, which may serve to target heterologous proteins to a variety of cellular membranes. In particular, the structural components of the nuclear envelope, or those components which become nucleus-associated, may be targeted with the sequences provided. Also provided are methods of targeting heterologous proteins to particular membranes, and the use of these targeted proteins in therapeutic, diagnostic and insecticidal applications.
DETD . . . production before, in 1970, severe disease outbreaks to fungal pathogens forced the industry to curtail its use. The URF13 protein (***13*** ***kDa***) encoded by the mitochondrial gene T-urfl13, is responsible for cms-T trait and has received considerable scientific investigation because of the. . .
DETD Hen, ***Borrelli*** , Fromental, Sassone-Corsi, and Chambon, "A Mutated Polyoma Virus Enhancer Which is Active in Undifferentiated Embryonal Carcinoma Cells is not Repressed. . .

L8 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2003 ACS
AN 1999:194170 CAPLUS
DN 130:236453
TI P13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination
IN Bergstrom, Sven
PA Symbicom Ab, Swed.
SO PCT Int. Appl., 118 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9912960	A2	19990318	WO 1998-IB1424	19980904
WO 9912960	A3	19990527		
W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2300365	AA	19990318	CA 1998-2300365	19980904
AU 9888811	A1	19990329	AU 1998-88811	19980904
EP 1012269	A2	20000628	EP 1998-940504	19980904
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI DK 1997-1041	A	19970910		

US 1997-59036P P 19970916
WO 1998-IB1424 W 19980904
AB A ***13*** ***kDa*** cell surface antigen (P13) found on Lyme disease ***Borrelia*** (B. burgdorferi, B. garinii, B. afzelii) but not B. hermsii, B. crocidurae, B. anserina, or B. hispanica and the gene for P13 are disclosed. Addnl., P13 epitopes, vectors, transformed cells, a method of prepg. P13 or P13 epitopes, and vaccines as well as diagnostic compns. and kits are further disclosed. The P13 genes of the 3 Lyme disease ***Borrelia*** were cloned and sequenced. The B. burgdorferi P13 gene was expressed in Escherichia coli.
TI P13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination
AB A ***13*** ***kDa*** cell surface antigen (P13) found on Lyme disease ***Borrelia*** (B. burgdorferi, B. garinii, B. afzelii) but not B. hermsii, B. crocidurae, B. anserina, or B. hispanica and the gene for P13 are disclosed. Addnl., P13 epitopes, vectors, transformed cells, a method of prepg. P13 or P13 epitopes, and vaccines as well as diagnostic compns. and kits are further disclosed. The P13 genes of the 3 Lyme disease ***Borrelia*** were cloned and sequenced. The B. burgdorferi P13 gene was expressed in Escherichia coli.
ST sequence Lyme disease ***Borrelia*** P13 antigen gene; vaccine Lyme disease ***Borrelia*** P13 antigen; diagnosis Lyme disease ***Borrelia*** PCR hybridization immunoassay
IT Antigens
RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)
(***13*** - ***kilodalton*** ; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT Antibodies
RL: ANT (Analyte); ANST (Analytical study)
(anti-Lyme disease ***Borrelia*** ; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT DNA sequences
(of P13 antigen genes of ***Borrelia*** burgdorferi, B. afzelii, and B. garinii)
IT Protein sequences
(of P13 antigens of ***Borrelia*** burgdorferi, B. afzelii, and B. garinii)
IT ***Borrelia*** afzelii
Borrelia burgdorferi
Borrelia garinii
Diagnosis
Epitopes
Escherichia coli
Molecular cloning
Nucleic acid hybridization
PCR (polymerase chain reaction)
Vaccines
(p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT Primers (nucleic acid)
Probes (nucleic acid)

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT Plasmids
(pLY313F; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT Plasmids
(pLY313T; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT 221180-87-6P 221180-88-7P 221180-89-8P 221180-90-1P
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(B. afzelii P13 epitope; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT 221180-81-0P 221180-82-1P 221180-83-2P 221180-84-3P 221180-85-4P 221180-86-5P
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(B. burgdorferi P13 epitope; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT 221180-92-3P 221180-93-4P 221180-94-5P 221180-96-7P 221180-97-8P
RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(B. garinii P13 epitope; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT 200654-83-7 221222-76-0 221222-78-2
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(amino acid sequence; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)
IT 221222-74-8 221222-75-9 221222-77-1
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process); USES (Uses)
(nucleotide sequence; p13 antigens and P13 genes of Lyme disease ***Borrelia*** and methods for diagnosis and vaccination)

L8 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2003 ACS
AN 1998:130561 CAPLUS
DN 128:204009
TI Characterization of the synovial T cell response to various recombinant Yersinia antigens in Yersinia enterocolitica-triggered reactive arthritis: heat-shock protein 60 drives a major immune response
AU Mertz, Andreas K. H.; Ugrinovic, Sanja; Lauster, Roland; Wu, Peihua; Grolms, Martina; Bottcher, Ute; Appel, Heiner; Yin, Zhinan; Schiltz, Emile; Batsford, Steven; Schauer-Petrowski, Christiana; Braun, Jurgen; Distler, Armin; Sieper, Joachim
CS Free University, Berlin, Germany
SO Arthritis & Rheumatism (1998), 41(2), 315-326
CODEN: ARHEAW; ISSN: 0004-3591
PB Lippincott-Raven Publishers

DT Journal
LA English
AB In Y. enterocolitica-triggered reactive arthritis (Yersinia ReA), the synovial T cell response is primarily directed against bacterial components, which are mostly unknown. This study was performed to investigate the synovial proliferative T cell response to a panel of recombinant Yersinia antigens in patients with Yersinia ReA and in controls. Synovial fluid mononuclear cells (SFMC) were obtained from 4 patients with Yersinia ReA and from 14 patients with arthritides of different etiol. SFMC were stimulated with 5 recombinant Yersinia antigens [the 19 kDa urease .beta. subunit, ***13*** ***kDa*** ribosomal L23 protein, 32 kDa ribosomal L2 protein, 18 kDa outer membrane protein H, and Y. enterocolitica heat-shock protein 60 (hsp60)], and with human, Chlamydia trachomatis, and ***Borrelia*** burgdorferi hsp60. Three T cell clones specific for Y. enterocolitica hsp60 were generated from 1 patient with Yersinia ReA. Antigen-induced cytokine release was measured by ELISA. SFMC from all 4 patients with Yersinia ReA responded to each of the Yersinia antigens except the ***13*** ***kDa*** protein. These antigens were also recognized by SFMC from a subgroup of patients with undifferentiated arthritis, but not by SFMC from other patients with arthritis of different etiol. Y. enterocolitica hsp60 induced the strongest proliferative response in all cases. Two types of hsp60-reactive T cell clones could be obtained. One clone responded to all hsp60 variants, including the human variant, and showed a type 2 T helper (Th2)-like cytokine-secretion pattern. In contrast, another clone with specificity for the bacterial hsp60 proteins, but not the human equiv., reacted with a more Th1-like pattern. In Y. enterocolitica-triggered ReA, at least 4 immunodominant T cell antigens exist, which might be used in lymphocyte proliferation assays to identify patients with Yersinia ReA. The hsp60 is a strong antigen, inducing both bacteria-specific and potentially autoreactive CD4+ T cells of both the Th1 and Th2 type.
RE.CNT 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
AB In Y. enterocolitica-triggered reactive arthritis (Yersinia ReA), the synovial T cell response is primarily directed against bacterial components, which are mostly unknown. This study was performed to investigate the synovial proliferative T cell response to a panel of recombinant Yersinia antigens in patients with Yersinia ReA and in controls. Synovial fluid mononuclear cells (SFMC) were obtained from 4 patients with Yersinia ReA and from 14 patients with arthritides of different etiol. SFMC were stimulated with 5 recombinant Yersinia antigens [the 19 kDa urease .beta. subunit, ***13*** ***kDa*** ribosomal L23 protein, 32 kDa ribosomal L2 protein, 18 kDa outer membrane protein H, and Y. enterocolitica heat-shock protein 60 (hsp60)], and with human, Chlamydia trachomatis, and ***Borrelia*** burgdorferi hsp60. Three T cell clones specific for Y. enterocolitica hsp60 were generated from 1 patient with Yersinia ReA. Antigen-induced cytokine release was measured by ELISA. SFMC from all 4 patients with Yersinia ReA responded to each of the Yersinia antigens except the ***13*** ***kDa*** protein. These antigens were also recognized by SFMC from a subgroup of patients with undifferentiated arthritis, but not by SFMC from other patients with arthritis of different etiol. Y. enterocolitica hsp60 induced the strongest proliferative response in all cases. Two types of hsp60-reactive T cell clones could be obtained. One clone responded to

all hsp60 variants, including the human variant, and showed a type 2 T helper (Th2)-like cytokine-secretion pattern. In contrast, another clone with specificity for the bacterial hsp60 proteins, but not the human equiv., reacted with a more Th1-like pattern. In Y. enterocolitica-triggered ReA, at least 4 immunodominant T cell antigens exist, which might be used in lymphocyte proliferation assays to identify patients with Yersinia ReA. The hsp60 is a strong antigen, inducing both bacteria-specific and potentially autoreactive CD4+ T cells of both the Th1 and Th2 type.

L8 ANSWER 17 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 1995:220154 BIOSIS
DN PREV199598234454
TI ***Borrelia*** burgdorferi mutant lacking Osp: Biological and immunological characterization.
AU Sadziene, Ariadna; Thomas, D. Denee; Barbour, Alan G. (1)
CS (1) Dep. Med., Univ. Texas Health Sci. Cent. at San Antonio, San Antonio, TX 78284 USA
SO Infection and Immunity, (1995) Vol. 63, No. 4, pp. 1573-1580. ISSN: 0019-9567.
DT Article
LA English
AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osps). Mutants of B. burgdorferi lacking Osps were selected with polyclonal or monoclonal antibodies at a frequency of 10-6 to 10-5. One mutant that lacked OspA, -B, -C, and -D was further characterized. It was distinguished from the OspA+B+ cells by its (i) autoaggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum and complement sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in mouse skin for the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two types of monoclonal antibodies (MAbs) with growth-inhibiting properties for mutant cells were identified. The first type bound to a ***13*** - ***kDa*** surface protein of B. burgdorferi sensu stricto and of B. afzelii. The MIC of the Fab fragment of one MAb of this type was 0.2 mu-g/ml. The second type of MAb to the Osp-less mutant did not bind to B. burgdorferi components by Western blotting (immunoblotting) but did not bind to unfixed, viable cells in immunofluorescence and growth inhibition assays. These studies revealed possible functions Osp proteins in ***borrelias*** , specifically serum resistance, and indicated that in the absence of Osp proteins, other antigens are expressed or become accessible at the cell surface.
TI ***Borrelia*** burgdorferi mutant lacking Osp: Biological and immunological characterization.
AB All ***Borrelia*** burgdorferi sensu lato isolates characterized to date have one or a combination of several major outer surface proteins (Osps). Mutants. . . Two types of monoclonal antibodies (MAbs) with growth-inhibiting properties for mutant cells were identified. The first type bound to a ***13*** - ***kDa*** surface protein of B.

burgdorferi sensu stricto and of B. afzelii. The MIC of the Fab fragment of one MAb. . . not bind to unfixed, viable cells in immunofluorescence and growth inhibition assays. These studies revealed possible functions Osp proteins in ***borrelias*** , specifically serum resistance, and indicated that in the absence of Osp proteins, other antigens are expressed or become accessible at. . .

ORGN . . .
Mammalia, Vertebrata, Chordata, Animalia; Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Spirochaetaceae: Eubacteria, Bacteria

ORGN Organism Name
human (Hominidae); mouse (Muridae); ***Borrelia*** burgdorferi (Spirochaetaceae)

ORGN Organism Superterms
animals; bacteria; chordates; eubacteria; humans; mammals; microorganisms; nonhuman mammals; nonhuman vertebrates; primates; rodents; vertebrates

L8 ANSWER 18 OF 19 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6

AN 1996:62220 BIOSIS

DN PREV199698634355

TI Chemiluminescent analysis of ***Borrelia*** burgdorferi penicillin-binding proteins using ampicillin conjugated to digoxigenin.

AU Norgard, Michael (1); Baker, Scott I.; Radolf, Justin D.

CS (1) Dep. Microbiol., Univ. Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235 USA

SO Microbial Pathogenesis, (1995) Vol. 19, No. 4, pp. 257-272. ISSN: 0882-4010.

DT Article

LA English

AB Knowledge of the penicillin-binding proteins (PBPs) of ***Borrelia*** burgdorferi is important for understanding both the targets of beta-lactams used therapeutically for Lyme ***borreliosis*** and the complex membrane biology of the distinctive spirochetal pathogen which causes Lyme disease. In this Study, the PBPs of a number of B. burgdorferi strains and variants were examined using a rapid and sensitive chemiluminescent assay which employs ampicillin conjugated to digoxigenin (dig-amp). The minimum inhibitory concentration of dig-amp for B. burgdorferi high-passage strain B31 (0.012 mu-g/ml) was essentially no different from that of free ampicillin (0.025 mu-g/ml). Dig-amp bound specifically to B. burgdorferi B31 PBPs with molecular masses of 92, 80, 65, 46, 40, 34, 31, 29, 22, 20 and ***13*** ***kDa*** ; the 31 kDa and 34 kDa PBPs were proven to be OspA and OspB, respectively. All of the ***borrelial*** PBPs were present in the cytoplasmic membrane fraction of B. burgdorferi, findings consistent with their activities as PBPs but inconsistent with OspA and OspB as surface-exposed outer membrane lipoproteins. Furthermore, among the PBP profiles of other high- and low-passage variants of B. burgdorferi strains Sh-2-82, HB19, and N40, which differed somewhat from one another, OspD (28 kDa) but not OspC (22-25 kDa) also was strongly implicated as a PBP; however, OspC possessed a gel mobility easily misconstrued as that of a 26 kDa PBP often expressed reciprocally with OspB. The ramifications of classifying OspA, OspS, and OspD as PBPs are discussed. While the current inability to genetically manipulate B. burgdorferi hinders determining which of the ***borrelial*** PBPs are essential for spirochetal viability (i.e., are

the lethal targets of beta-lactams), a priori knowledge of the ***borrelial*** PBPs will facilitate the production and purification of recombinant derivatives whose activities can be assessed further in vitro.

TI Chemiluminescent analysis of ***Borrelia*** burgdorferi penicillin-binding proteins using ampicillin conjugated to digoxigenin.

AB Knowledge of the penicillin-binding proteins (PBPs) of ***Borrelia*** burgdorferi is important for understanding both the targets of beta-lactams used therapeutically for Lyme ***borreliosis*** and the complex membrane biology of the distinctive spirochetal pathogen which causes Lyme disease. In this Study, the PBPs of. . . to B. burgdorferi B31 PBPs with molecular masses of 92, 80, 65, 46, 40, 34, 31, 29, 22, 20 and ***13*** ***kDa*** ; the 31 kDa and 34 kDa PBPs were proven to be OspA and OspB, respectively. All of the ***borrelial*** PBPs were present in the cytoplasmic membrane fraction of B. burgdorferi, findings consistent with their activities as PBPs but inconsistent. . . and OspD as PBPs are discussed. While the current inability to genetically manipulate B. burgdorferi hinders determining which of the ***borrelial*** PBPs are essential for spirochetal viability (i.e., are the lethal targets of beta-lactams), a priori knowledge of the ***borrelial*** PBPs will facilitate the production and purification of recombinant derivatives whose activities can be assessed further in vitro.

IT Miscellaneous Descriptors
ANALYTICAL METHOD; BETA-LACTAM; LYME ***BORRELIOSIS*** ; OUTER MEMBRANE PROTEIN

ORGN Super Taxa
Spirochaetaceae: Eubacteria, Bacteria

ORGN Organism Name
Borrelia burgdorferi (Spirochaetaceae)

ORGN Organism Superterms
bacteria; eubacteria; microorganisms

L8 ANSWER 19 OF 19 CABA COPYRIGHT 2003 CABI

AN 92:127213 CABA

DN 920511829

TI Polymorphism on outer surface proteins of ***Borrelia*** burgdorferi as a tool for classification

AU Peter, O.; Bretz, A. G.

CS Clinical Microbiology, Institut Central des Hopitaux Valaisans, Case postale 510, CH-1951 Sion 3, Switzerland.

SO Zentralblatt fur Bakteriologie, (1992) Vol. 277, No. 1, pp. 28-33. 19 ref.

DT Journal

LA English

SL German

AB A total of 23 isolates of B. burgdorferi were characterized by SDS-PAGE and immunoblot analysis. One isolate came from the CSF of a Lyme neuro-***borreliosis*** patient in Valais, Switzerland, and 22 were tick isolates (2 from Ixodes dammini of Shelter Island, New York, USA, and 20 from I. ricinus of Valais, Switzerland). Based on the electrophoretic mobility of outer surface proteins (OspA and OspB), 4 groups of B. burgdorferi could be defined. Group I isolates possess an OspA of 31 kDa and an OspB of 34 kDa. The group II isolate showed an OspA of 32 kDa and OspB of 35 kDa. Group III isolates have a 33-kDa OspA and group IV a 33.5-kDa OspA. This classification was confirmed by the reactivity of a monoclonal antibody (D6) to a 12-kDa antigen that was recognized in group III only. A Lyme patient's serum showed a 2-band pattern (10 and

13 ***kDa***) for group I and a one-band pattern (12 kDa) for the other 3 groups. Therefore OspA, OspB and other proteins of low molecular weight (10, 12 and ***13*** ***kDa***) seem to be important keys for the classification of B. burgdorferi isolates. This typing system correlates with genetic analysis.

TI Polymorphism on outer surface proteins of ***Borrelia*** burgdorferi as a tool for classification.

AB . . . isolates of B. burgdorferi were characterized by SDS-PAGE and immunoblot analysis. One isolate came from the CSF of a Lyme neuro-***borreliosis*** patient in Valais, Switzerland, and 22 were tick isolates (2 from Ixodes dammini of Shelter Island, New York, USA, and. . . a 12-kDa antigen that was recognized in group III only. A Lyme patient's serum showed a 2-band pattern (10 and ***13*** ***kDa***) for group I and a one-band pattern (12 kDa) for the other 3 groups. Therefore OspA, OspB and other proteins of low molecular weight (10, 12 and ***13*** ***kDa***) seem to be important keys for the classification of B. burgdorferi isolates. This typing system correlates with genetic analysis.

BT Spirochaetales; Gracilicutes; bacteria; prokaryotes; ***Borrelia*** ; Spirochaetaceae; Western Europe; Europe

ORGN Spirochaetaceae; ***Borrelia*** burgdorferi